

SYMBOLAE BOTANICAE UPSALIENSES X:1

ARBETEN FRÅN BOTANISKA INSTITUTIONERNA I UPPSALA

UTGIVNA AV PROF. E. MELIN OCH PROF. J. A. NANNFELDT

ENVIRONMENTAL REQUIREMENTS OF FRESH-WATER PLANKTON ALGAE

EXPERIMENTAL STUDIES
IN THE ECOLOGY OF PHYTOPLANKTON

BY

WILHELM RODHE



UPPSALA

A.-B. LUNDEQUISTSKA BOKHANDELN

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Preface.

The productivity of lakes has long been, and will always remain, in the centre of limnological research. In productivity problems the development of phytoplankton, and also its relations to the environmental conditions, possess a dominating place. Consequently, our understanding of lake productivity presupposes, among other things, a good knowledge of the environmental requirements of plankton algae.

These questions may be studied in two different ways: by ecological work on the biotopes themselves and by physiological investigations under experimental conditions. Neither of these ways alone, however, leads to the final aim. To reach this, a combination of ecological and physiological methods and data is necessary.

In the summer of 1933, I began to follow the first line of investigation under the stimulating guidance of the late Professor EINAR NAUMANN. During the following summers, I studied the environmental conditions of some lakes in the vicinity of the limnological laboratory at Aneboda in southern Sweden. In 1937 and 1938, these studies were intensified in collaboration with Dr BÖRJE ÅBERG, resulting in a joint work published in 1942. My investigations of the phytoplankton in the Aneboda lakes, which were planned to follow, could not, however, be carried out.

For this reason I began, in the summer of 1940, similar studies in a number of lakes in the neighbourhood of Uppsala. As before, my first task was to carry out physical and chemical investigations in order to get information on the environments in which the algae lived. In the summer of 1943, after three years' observations of the biotopes, I took up simultaneous quantitative studies of the dominating plankton algae. Within a few weeks, however, this work was interrupted by military duties.

After that I laid the main stress of my phytoplankton studies on an experimental basis with pure cultures of different species. The field investigations were certainly continued, but in a less intensive manner. Opportunities of a direct combination of ecological and physiological aspects were given — thanks to a testamentary donation by CARL MALMÉN

to the University of Uppsala — by the foundation of a limnological laboratory at the lake Erken in August 1944. Consequently this lake and its phytoplankton obtained a yet more prominent position in my work than before.

The arrival of Professor HEINRICH SKUJA in Uppsala, at the beginning of 1945, proved to be of great importance for my investigations. From taxonomic points of view, he first studied the preserved phytoplankton samples, gathered from ten lakes during 1940—1944. During the two following years he subjected the plankton algae of these lakes to a thorough and successful examination, carried out on living samples immediately after their transport from the biotopes. Besides the taxonomic investigations, which are now published in an extensive monograph, SKUJA has compiled a great number of most complete tables showing the (relative) frequencies of the different algae in the lakes investigated. These algal lists of SKUJA, and my own corresponding environmental data, are to be published together in the near future. Some information available from this material is, however, already utilized here in connection with my experimental results.

From what has been said, it is evident that the purpose of the experimental studies presented in this book has been ecological rather than physiological. This may explain and excuse my treatment of the problem, which treatment has in many cases been rather superficial from a physiological point of view. In ecological questions it is often enough to state how an organism reacts to different environmental conditions, since such statements can be used as explanations of the complex relationships in nature. It is a purely physiological task to explain why the organism reacts in just the manner stated.

*

The present investigations have been mainly carried out at the Institute of Physiological Botany of the University of Uppsala. A part of the work has, however, been done at the Erken Limnological Laboratory. To their Director, Professor ELIAS MELIN, I convey my warmest thanks for the valuable support and the great interest which he has always given to me and my investigations.

To Professor HEINRICH SKUJA I express my deep thanks for placing his exceptional practical knowledge concerning the taxonomy of the plankton algae at my disposal.

For fruitful discussions and valuable advice, I thank Professor JOHN AXEL NANNFELDT and Professor G. EINAR DU RIETZ.

Since May 1945, I have had a very skilful laboratory assistant in Mrs

IRMA SLANKIS, who has played a very great part in obtaining the results submitted here. I thank her sincerely for her careful and interested work. Also to Mr HALVOR ØSTTVEIT, Cand. Mag., who assisted in the laboratory work during 1944, I convey my thanks. Similarly, I thank Mr ÅKE DANIELSSON, Laboratory Steward at this Institute, who has facilitated my work in many ways during the last eight years.

Docent GUNNAR LOHAMMAR has followed my investigations right from the beginning with a stimulating interest. I thank him and Mr SVEN ÖSTERLIND, Fil. Lic., for valuable discussions and appreciated criticism.

The translation of this work has been carried out by Mr EDWARD J. BURGE, B.Sc., who also, together with Mr F. J. TAYLOR, B.Sc., shared in the proof-reading. To both I convey my best thanks.

Without the aid of grants from different funds, my investigations could not have been accomplished. The expenses connected with the field-work and the travelling thereby involved have been particularly heavy. Contributions have been received from various funds of Uppsala University (Liljewalchs and Thuns stipendiefonder and Malménska studiefonden) and of the Royal Swedish Academy of Sciences (Hierta-Retzius' stipendiefond, Hierta-Retzius' fond för vetenskaplig forskning, Per Erik Lindahls stipendiefond), and from Stiftelsen Lars Hiertas Minne and Naturvetenskapliga Studentsällskapets Linné-stipendiefond. During the final period of the preparation of this dissertation I have obtained help by a State Grant for Advanced Studies (doktorandstipendium). For all this support I express my warm gratitude.

Uppsala, Institute for Physiological Botany. April 1948.

WILHELM RODHE.

I. Determination of Growth and Chlorophyll Content in Pure Cultures of Plankton Algae.

Introduction.

The cultivation of algae in pure cultures can give information on the processes of life and the environmental requirements of the cultivated organisms. They are therefore valuable aids in studies of the physiology and ecology of algae. Towards this end, much attention has been given to algal cultures of different kinds since the end of the last century. The extensive literature in this field has recently been reviewed and commented on by BOLD (1942). PRINGSHEIM (1946) has, yet later, summarized his great technical experiences with particular reference to algae which are difficult to cultivate and of mixotrophic character.

The euplanktic algae were earlier often considered to be particularly difficult or quite impossible to cultivate in synthetic culture media. The unsatisfying or negative results in the culture of plankton algae depended, however, mainly on the fact that sufficient observance had not been given to the conditions in the natural biotopes, which should be guides for the compositions of the culture media (cf. NAUMANN 1929 p. 80). An examination of the 140 odd culture formulae for fresh-water algae, which KUFFE-RATH gathered together in 1929, shows that nearly all the solutions and substrates proposed up to then, both with regard to quality and quantity, diverge widely from the chemical composition and total salt content of natural waters.

The first investigator who purposely tried and succeeded to cultivate fresh-water plankton algae in completely synthetic and bacteria-free solutions was CHU (1942). By a suitable choice of the composition of the culture solutions in the region of the concentrations in natural waters, he obtained large growths of several euplanktic green algae and diatoms. He found (p. 297) that the photometric measurement of the light absorption of the cultures is not only simpler and quicker than the determinations of the number, area (volume), or dry weight of the algae, but that this method also gives a more certain and better estimation of growth. In the cases

where the size of the individuals varies in different parallel cultures, or in the same culture on different occasions, determinations of their number or area per unit volume of culture solution give a false picture of the growth. Therefore CHU used, without reserve, in the first place the photometrically obtained extinction values as a measure of the growth in the cultures.

GROSS & KOCZY (1946), obviously without knowledge of the work of CHU, have studied the connection between extinction (determined with the Pulfrich photometer using a suitable colour filter) and the number of cells in pure cultures of marine plankton algae. They considered it possible to state (p. 7) that "the extinctions of all concentrations used lie on a straight line, indicating that they are linearly proportional to the number of cells." However, they found that it was not possible, even for one and the same species, to construct a reproducible standard curve, whilst with the same number of cells variations up to 20 % of the value of the extinction coefficient was caused by probable physiological differences between the algal material of the cultures.

Since the beginning of 1944 I have, to a large extent, used the photometrically obtained extinction values for establishing the growth of different plankton algae (mostly green algae but also diatoms) in pure cultures. Side by side with the photometric determinations, I have often made, not only counts of the number of cells or colonies per mm^3 , but also determinations of the chlorophyll content of the cultures. In the following an account is given of the methods used and some results of interest concerning these methods.

Methods.

A. Photometric determination of algal cultures.

The photometer constructed by ÅBERG & RODHE (1942 pp. 80—87), with a plane-parallel cuvette (100 mm high, open area 50×50 mm) placed between the light source and the photocell as in the arrangement recommended by CHU (p. 296, fig. 5), could easily be used for the photometric determination of algal cultures in tubes. The culture tubes used, 200 mm long and with an outer diameter of 18—20 mm, were of Jena glass and had a coloured line along their lengths. This mark was placed in the line of sight between the scratches on two screws in the lid of the cuvette on each side of the tube, by which means the tube could be kept in the same position with respect to the beam of light for each measurement.

For the photometric determination, the light intensity was first adjusted by a variable resistance so that the galvanometer showed the

greatest possible deflection (I_0). The tube was then placed in position and the deflection became I . The extinction (e) can be calculated by the formula

$$e = \log \frac{I_0}{I}. \quad (1)$$

The absolute extinction coefficient (E) can be obtained from this value, if so desired, by dividing by the 'effective' layer-thickness of the tube (d cm)

$$E = \frac{e}{d}. \quad (2)$$

In the beam of light (5 mm wide and 20 mm high) the effective thickness of the tubes used varied between 1.79 and 1.85 cm. Within each series of experiments I have aimed at as great conformity as possible in the outer diameter of the tube. On the other hand, rather large differences have occurred between different series. The extinction values for different tubes can have an error amounting to about 3 % depending on their different thicknesses.

After inoculation, the extinction value of the culture tube was determined for the start of the experiment (e_0). This value was then subtracted from the extinction values ($e_1, e_2 \dots e_v$) obtained during the experiment. Each difference ($e_v - e_0$) is then an expression for the change in the light absorption of the culture due to the algae. Because the extinction value is usually less than unity, and must even in other cases be expressed in decimals, I prefer, for the sake of simplicity, to give the difference multiplied by 1,000, thus

$$Z = (e_v - e_0) \times 10^3. \quad (3)$$

B. Counting of cells (colonies).

A blood-counter according to THOMA or BÜRKER can generally be used directly for the counting of the number of individuals or colonies per unit volume. Concerning their use I refer to medical textbooks, e. g. MÜLLER (1924) or SCHILLING (1942). Only a few words may be said here concerning the statistical treatment of the values.

From each sample I usually take at least two drops which are counted separately, and for each drop I count the number of cells (colonies) in at least three equally large areas, whose size (a mm², a usually < 1) is chosen with regard to the population density in question. In this way a

number (n , $n \geq 6$) of numerical values (x_v) is obtained for which the standard error (ϵ) of the mean ($\bar{x}$) is calculated from

$$\epsilon(\bar{x}) = \pm \sqrt{\frac{\sum (x_v - \bar{x})^2}{n(n-1)}}.$$

The mean with its standard error holds, of course, for the chosen area ($a \text{ mm}^2$). Since the depth of the counting chamber is 0.1 mm, the values corresponding to 1 mm³ are obtained by multiplication of $\bar{x}$ and $\epsilon(\bar{x})$ by 10/ a .

C. Determination of chlorophyll content.

At room temperatures the chlorophyll of living cells is only extracted slowly and incompletely by methyl alcohol. At the boiling-point of methyl alcohol (65° C) the extraction goes quickly and completely. On the other hand, chlorophyll is not stable in warm methanol, whilst it is fairly stable in methyl alcohol at room temperatures. These relations are decisive for the procedure used here (cf. FLEISCHER 1935 p. 585).

10 ml of algal culture solution were centrifuged (3,500 revs/min., 8 cm radius to liquid surface) for a few minutes, after which the cell-free solution could be decanted. 7—8 ml of methanol and a glass pebble were added and the tube placed in a water-bath at about 90° C. When, after about a minute, the alcohol began to boil, the tube was taken out and only dipped into the water-bath now and then in order to maintain even boiling (the glass pebble prevents 'bumping'). After exactly one minute's boiling all the contents of the tube were poured onto a porcelain filter, placed in the mouth of an evacuable bell-jar and with the stem of the funnel going down into a measuring-flask of 11 ml. The solution was quickly suction-filtered into the measuring-flask, after which the algal residue on the filter was washed with a few ml of methyl alcohol which were allowed to mix with the solution in the flask. The evacuation was stopped, the measuring-flask taken out from the bell-jar and cooled in cold water. Methyl alcohol was added up to the 11 ml mark and the sample was then ready for the photometric measurement.

This was carried out in a tube cuvette of 50 mm effective thickness and a little more than 10 ml in volume; since the volume of the sample was 11 ml there was nearly 1 ml of the extract available to rinse the cuvette after a previous measurement. Colour filter RG 1 (Schott und Gen., Jena; for the optical properties see ÅBERG & RODHE 1942 p. 11) was used to eliminate the effect of carotins. The photometric measurements were made with the same apparatus as was used for the extinction determinations of

algal cultures, with a special holder for the 5 cm cuvette. Calculation of the extinction value was made according to formula (1) given above. The blank values of the cuvette and the methanol were not subtracted, since all the measurements were made in the same manner. The obtained total value is indicated as $K (= e \times 10^3)$.

In order to make possible the transformation of the K values to weights of chlorophyll, a calibration curve was established in the following way. 50 mg of a chlorophyll preparation ("Chlorophyll puriss." from Kebo, Stockholm) were dissolved in 100 ml of methanol and this solution (A) was compared colorimetrically with an artificial chlorophyll standard according to GUTHRIE (1928), prepared with CuSO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$, and NH_4OH in certain proportions. According to this determination, solution A contained 196 mg of chlorophyll per litre (instead of 500 mg/l according to the label of the preparation!). By dilution of solution A with methyl alcohol, a series with concentrations rising in steps from 1 to 20 mg of chlorophyll per litre ($= 10$ to $200 \mu\text{g}$ per 10 ml sample) was prepared. In order to get agreement with the analytical procedure 10 ml of solution + 1 ml of methanol were measured photometrically using the same cuvette and colour filter as was used for the chlorophyll determinations themselves. The values obtained are given together with the resulting calibration curve in fig. 1.

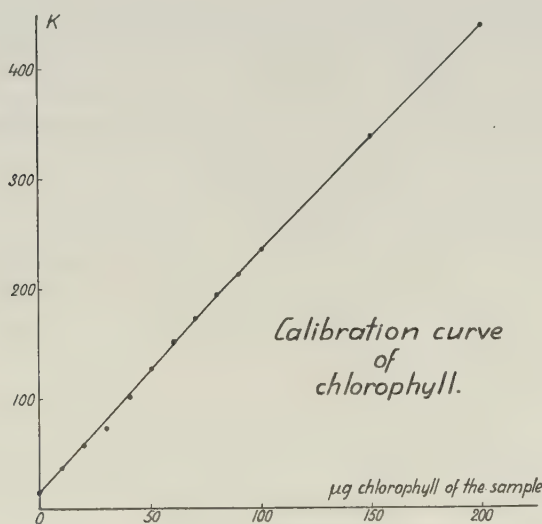


Fig. 1.

Calibration curve for chlorophyll determinations with colour filter RG 1 and a 50 mm cuvette.
 $K = e \times 10^3$.

From the point of intersection with the y -axis it follows that about 15 units correspond to the extinction of the cuvette and the methyl alcohol.

Extinction value, cell concentration, and substance production.

As has already been mentioned in the introduction, GROSS & KOCZY on the basis of dilution experiments considered that different cell concentrations give a straight line for the corresponding extinction values. By the following experiment with the green alga *Ankistrodesmus falcatus*, I have come to a diverging result.

110 ml of culture solution according to CHU's recipe No. 10 (1942 p. 298) and 11 ml of soil extract were placed in each of two conical flasks (104 a and 104 b) of 200 ml. The flasks were autoclaved and inoculated, each with 10 drops from a highly productive culture of *Ankistrodesmus* in the same culture solution. 10 ml were taken out from each solution to two culture tubes which were photometrically measured to obtain the e_0 -value; these tubes were then used for the measurements of the algal cultures. Flask 104 a was placed in a photothermostat (cf. ÅBERG 1943 p. 28) at 25° C with 10 hours' illumination per day; flask 104 b was placed for the first 12 days in diffuse daylight at room temperatures and then transferred to the photothermostat.

The growth was good in both the cultures, but it was quite obvious that culture 104 a in the photothermostat grew somewhat more quickly than culture 104 b in daylight at room temperatures. The former culture was stopped 12 days after inoculation ($Z = 583$), whilst culture 104 b was used four days later, during which time in the photothermostat its population density rapidly increased (to $Z = 770$).

By stepped dilution of 104 a and 104 b, two series of solutions were obtained with different concentrations of *Ankistrodesmus*. Counts in the blood-counter of both 104 a and 104 b as well as of some different dilutions gave an average of 8,820 cells/mm³ for 104 a, and 12,750 cells/mm³ for 104 b. The curves obtained by photometric measurements of both series of dilutions are given in fig. 2.

It is obvious that the relation between the number of *Ankistrodesmus* cells per mm³ (A) and the corresponding extinction value (Z) is not linear but is represented by a slightly curved line. This becomes yet more obvious if, with the guidance of the curve, one calculates the quotient of both the variables, which must of course be constant for a linear correlation in the whole range of concentration. The quotients are made clear in fig. 3, expressed as $10 Z/A$. As appears, the value of the quotient depends on the concentration, retracing a maximum curve for increasing extinction.

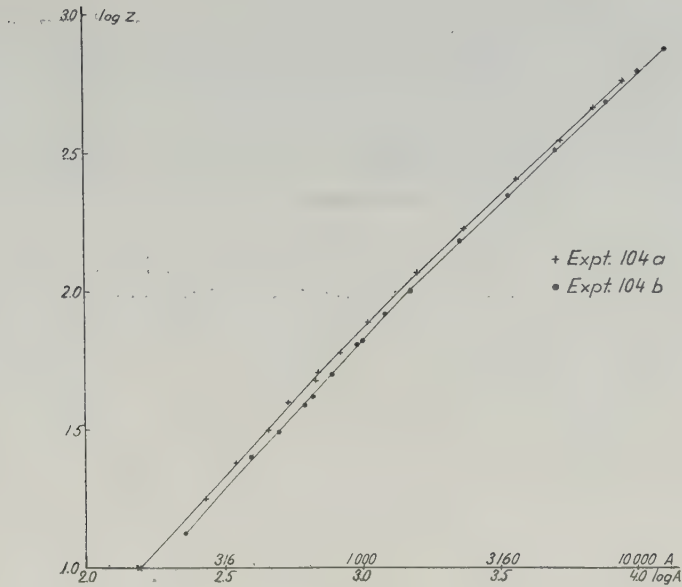


Fig. 2.

Relation between extinction values (Z) and cell numbers (A) in different dilutions of two cultures of *Ankistrodesmus falcatus*.

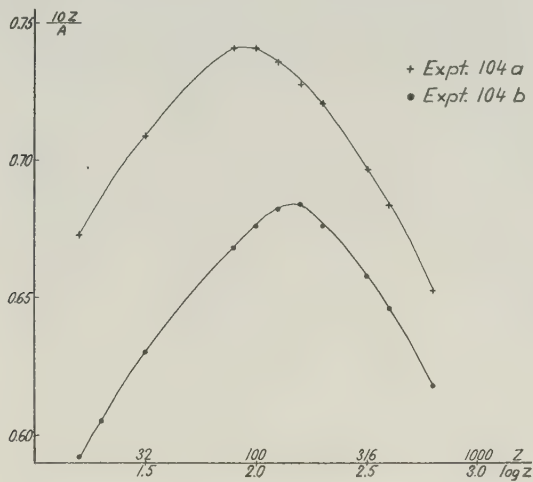


Fig. 3.

Relation between the gradients (Z/A) and the extinction values (Z) of the curves in fig. 2.

This is in fact a natural and, from colorimetry, well-known fact: LAMBERT-BEER'S Law, according to which the extinction coefficient is a linear function of the concentration of the light-absorbing substance, only holds rig-

idly for monochromatic light. For white light or coloured non-monochromatic light (e. g. such as is obtained with most colour filters) the function is most often more complicated, because the dependence of the light absorption on concentration is not the same for different wavelengths. The consequence is usually that the relation of the extinction coefficient (composed of different constants) to the concentration is expressed as a more or less bent curve.

If one examines the linear extinction curves constructed by GROSS & KOCZY more closely, it appears, at least for *Skeletonema* (see loc. cit., fig. 2), that the values obtained are more inclined to give a curved line from the origin than the straight line drawn which intersects the y -axis above the origin. Probably "Flagellate D" also would prove, on closer investigation, to have a slightly bent extinction curve.

In practice this divergence from direct proportionality (which is to be expected for all extinction determinations with white light or light which has passed through a colour filter¹) ought not to be so important that it jeopardizes the applicability of such determinations. It should still be held in mind for comparisons of extinction values for different concentrations. The source of error should be able to be eliminated completely if, with the help of a calibration curve, one could transpose the extinction values to the corresponding absolute concentration values. For different concentrations of one and the same algal solution this is, as shown above in fig. 2, easy to arrange, but for the most urgent need in this connection, namely the determination of growth in a culture, we meet difficulties of a completely different kind.

We first notice that the extinction curves of fig. 2 have somewhat different slopes and that the corresponding quotient curves in fig. 3 are also different, both concerning the magnitude of the quotient and its dependence on concentration. These differences could scarcely be explained by experimental errors, e. g. in the determination of the number of cells in 104 a and 104 b respectively, but must depend on the fact that the material in the two cultures is not identical with regard to the character of the cells. It will be remembered that the cultures had different conditions for a large part of the time of the experiment. The importance of the outer conditions for the extinction curve of the culture is clearly evident from the following experiment.

For the investigation of the importance of light intensity and tempera-

¹ Since this was written, an investigation by VAN GENDEREN & SCHOLTENS (1947) has shown that a linear correlation is obtained for bacterial cultures, if monochromatic light is used.

ture for the growth of *Ankistrodesmus falcatus*, *Scenedesmus acutus*, and a strain of *Chlorella* (experiment 102, cf. p. 136), a number of pure cultures of these algae had been placed in Jena tubes with 10 ml of culture solution Chu 10 + 10 % soil extract in photothermostats at 15° C, 20° C, and 25° C, at different distances from the light source with illumination from 8 a. m. to 6 p. m. each day. Photometric determinations were made regularly from 9 to 10 a. m. but for a short time (20th—24th of March) the cultures were also measured from 5 to 6 p. m., i. e. at the end of the illumination time; each culture was withdrawn for 10 minutes at the most from the photothermostat for measurement.

By photometric measurements both at the beginning and the end of the period of illumination a rhythm in the extinction curve involved with the light-darkness periodicity could be observed for all cultures. For *Ankistrodesmus* cultures 102 A: 1, A: 2, and A: 3 (15° C, light intensity A: 1 > A: 2 > A: 3) this is made clear in fig. 4.

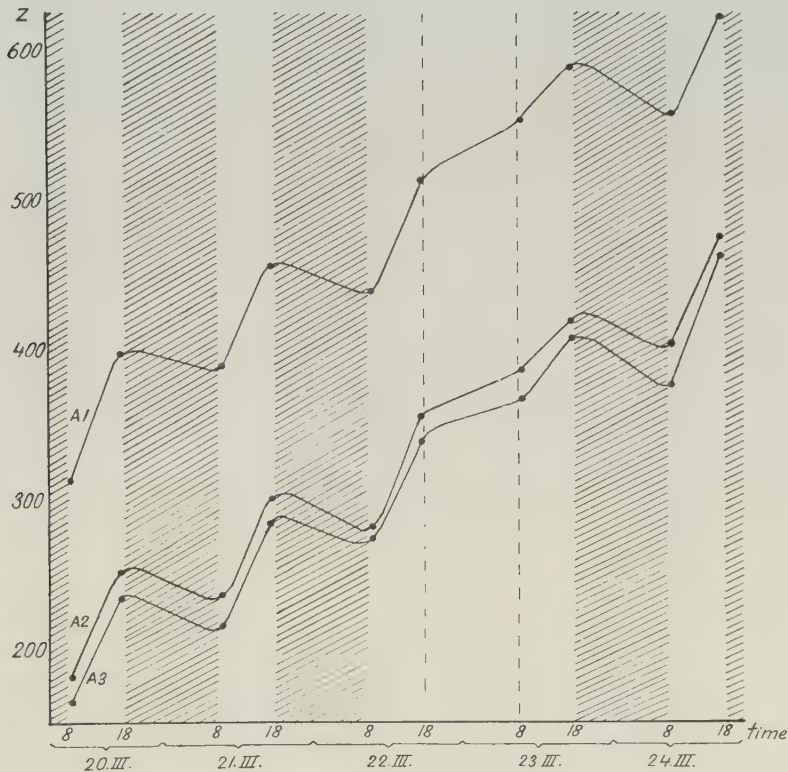


Fig. 4.

Dependence of the extinction values (Z) of *Ankistrodesmus* cultures on light-dark periods.

Between the 22nd and 23rd of March the illumination was continued even during the hours (6 p. m.—8 a. m.) when otherwise a dark period took place. The curves show, however, a course which, despite the constant external conditions, is reminiscent of the growths in connection with the earlier light-darkness periods. The light-induced rhythm has obviously been to a certain extent involved endogenously.

As follows from fig. 4, the extinction values decrease during the dark period. A diminution of the number of cells is, however, excluded. On the contrary one should be able to assume that — as is often the case in nature during the night — a specially lively cell-division takes place during or at the end of the dark time. Obviously the positive correlation between extinction and cell-number has here been completely destroyed by other factors.

The size of the cells can scarcely come into the question as such a factor. The only thing that remains is the substance content. It is easy to imagine that the assimilates built up during the light period in the form of starch are stored in a solid form in order to be hydrolyzed and transferred to soluble sugars during the dark period. This must involve an increase of the solid substance of the culture in light and a decrease in darkness. It appears not improbable that such a reaction which is dependent on enzymes and enzyme concentrations in the cell, can continue to a certain extent, at least for a short time, when the original impulses disappear. The interesting physiological questions which this photoperiodicity with three green algae provokes could not be treated more closely here. It appears that the problem is very likely to be well worth a separate investigation.

The extinction values given in fig. 4 are obviously in the first place an expression for the solid substance content of the cultures. Since the production of substance is dependent on the light-dark periods, among other things, it is necessary in order to obtain comparable extinction values to make photometric determinations at times which are equivalent in relation to these periods. Otherwise misleading growth curves could be obtained.

Since the cell number is not the primary determinant for the course of the extinction curve of a growing culture, it appears impossible, even for one and the same species and strain, to evaluate directly from the extinction value the exact number of cells per unit volume. In this respect CHU (1942 p. 297) has overestimated the applicability of the photometric method. At the most, for identical conditions, one should be able to obtain approximate values for the cell number with the help of a calibration curve drawn for these conditions.

Growth and chlorophyll formation.

To 500 ml of my culture solution VIII (see below p. 57), 1 ml of a deep-green pure culture of *Scenedesmus quadricauda* in the same medium was added. 200 ml of the inoculated solution were placed in each of two 300 ml flasks (913 A and 913 B respectively), which were placed in a photothermostat at 25° C with illumination from 3 p. m. to 11 a. m. (20 hours out of 24). The number of cells of *Scenedesmus* (in four-cell colonies) at the start was less than 1 per mm³ and no colour or cloudiness could then be observed with the naked eye.

During the experiment simultaneous double determinations were made of the extinction value (Z), the chlorophyll content (K), and the cell number per mm³ (A). For this purpose 2×10 ml were taken from each flask at each sampling. After photometric measurement and the withdrawal of a few drops for cell counting in the BÜRKER chamber, these samples were centrifuged for the determination of chlorophyll. In order to give an idea of the reliability of the individual values they are shown altogether (with their averages) in Tables 1 A and 1 B. Figs 5 A and 5 B give a graphical presentation of the results of the experiment (averages), in which the cell number is divided by 10 so that all three curves could be given on the same scale.

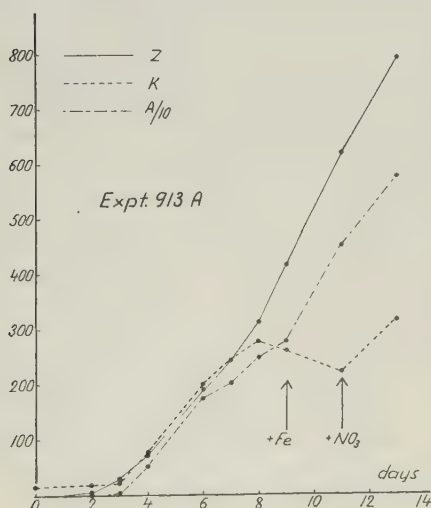


Fig. 5 A.

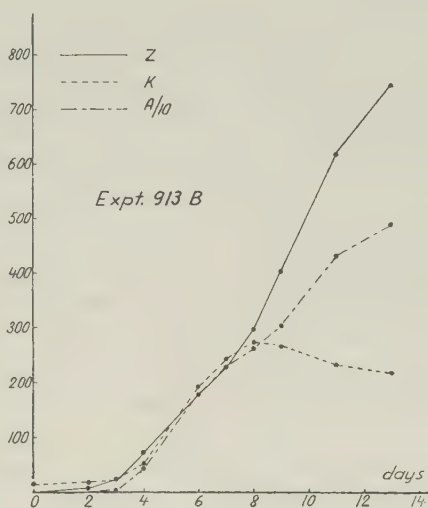


Fig. 5 B.

Mutually independent time-curves of extinction values (Z), chlorophyll contents (K), and cells/mm³ (A), for two parallel cultures of *Scenedesmus quadricauda*.

Table 1. Expts 913 A and 913 B. *Scenedesmus quadricauda*. Record of growth by determinations of extinction, chlorophyll, and cell number.

A. Experiment 913 A.

Days	Extinction value, Z		Chlorophyll content, K		Cell number per mm ³ , A	
0	0	0	15		< 1	
	0		15	15	< 1	< 1
2	5		18		8	
	—	5	18	18	11	9
3	27		22		40	
	29	28	24	23	55	48
4	73		76		593 $\pm$ 86	
	71	72	76	76	468 $\pm$ 97	531
6	194		199		1,600 $\pm$ 87	
	188	191	201	200	1,900 $\pm$ 124	1,750
7	245		244		2,110 $\pm$ 108	
	243	244	247	246	1,930 $\pm$ 123	2,020
8	314		278		2,640 $\pm$ 180	
	311	312	276	277	2,340 $\pm$ 168	2,490
+ Fe 9	422		260		2,740 $\pm$ 193	
	416	419	263	261	2,820 $\pm$ 150	2,780
+ NO ₃ 11	621		222		4,520 $\pm$ 171	
	—	621	—	222	—	4,520
13	798		319		5,850 $\pm$ 264	
	797	797	319	319	5,720 $\pm$ 137	5,785

Only two days after the start the growth was quite obviously much increased and measureable by all three methods. During the following days of the experiment the extinction value, chlorophyll content, and cell number show a rapid and consistent increase. The approximate numerical similarity between Z , K , and $A/10$ for this stage of growth is of course accidental (extinction and chlorophyll determinations with different layer-thicknesses than those used here would give other values for Z and K), but it is advantageous from a practical point of view.

In both experiments the chlorophyll content reached its maximum on the eighth day. That the formation of chlorophyll ceased and gave place to a reverse process (autolysis) was then very clearly noticed by the shade of yellow which made itself evident in the earlier fresh green of the culture.

A solution of ferric citrate — citric acid was added to 913 A on the ninth day, to the extent that the Fe-content of the culture was increased by the original dose (0.18 mg Fe/l). As follows from the measurements of the eleventh day, this had no effect on the formation of chlorophyll; on the contrary the chlorophyll content continued to decrease in both cultures.

B. Experiment 913 B.

Table 1 (cont.)

Days	Extinction value, Z		Chlorophyll content, K		Cell number per mm ³ , A	
0	0	0	15	15	< 1	< 1
	0		15		< 1	
2	7	7	18	18	10	10
	—		18		11	
3	21	22	24	23	48	44
	22		22		40	
4	70	71	56	54	420 $\pm$ 58	444
	72		52		468 $\pm$ 60	
6	178	179	190	192	1,860 $\pm$ 170	1,780
	180		194		1,700 $\pm$ 121	
7	224	227	242	241	2,550 $\pm$ 341	2,305
	231		239		2,060 $\pm$ 227	
8	294	296	276	272	2,640 $\pm$ 132	2,640
	298		268		2,640 $\pm$ 214	
9	404	402	276	268	3,130 $\pm$ 150	3,045
	401		260		2,960 $\pm$ 250	
11	616	616	232	232	4,300 $\pm$ 180	4,300
	—		—		—	
13	747	744	222	217	4,720 $\pm$ 338	4,890
	742		212		5,060 $\pm$ 292	

Then $\text{Ca}(\text{NO}_3)_2$, corresponding to the original concentration (10 mg N/l), was added to 913 A and after only a few hours it could be observed that this culture had a greener colour than 913 B. The recommencement of the formation of chlorophyll also appears in the increase of K for 913 A on the thirteenth day. The formation of chlorophyll clearly ceased because of the lack of nitrate (cf. p. 93).

In none of the experiments were these essential changes in the chlorophyll formation and content reflected in the extinction curve, which shows a fairly constant rise from the third day of the experiment. As a light absorbing substance, chlorophyll obviously has little importance compared with the other factors affecting the extinctions of algal cultures.

The result obtained above, that neither does the cell number in a growing culture present a primary factor for the extinction, is confirmed by experiment 913. It certainly appears that the cell division — like the substance production — also continues after the onset of the lack of nitrate (a fact which is of ecological interest), but it appears as if the rate of division is, to some degree, affected by this deficiency.

The following values of the quotient $10 Z/A$ could be obtained from the average values in Tables 1 A and 1 B:

Day	913 A	913 B
2	5.6	7.0
3	5.8	5.0
4	1.4	1.6
6	1.1	1.0
7	1.2	1.0
8	1.3	1.1
9	1.5	1.3
11	1.4	1.4
13	1.4	1.5

In both experiments, the value of the quotient traces a minimum curve when the cell concentration increases. This relation cannot be connected with the divergences from LAMBERT-BEER's Law found above (p. 14), which gave a maximum curve for the value of the quotient. Consequently it is obvious that the average substance content per cell varies with the age of the culture: during the first six days of the experiment the amount of substance per cell decreased, after which it gently increased. This course agrees with the results found by PEARSALL & LOOSE (1937) with *Chlorella vulgaris*, according to which "the dry weight per unit volume of cell material . . . appears to be minimal at, or just after the end of, exponential growth" (p. 496). This factor alone makes a general conversion of the extinction value to the cell number very doubtful.

The photometric measurement of algal cultures has, however, an independent value as a quick and reliable method for the quantitative following of their substance production. The extinction measurements alone, however, could not give sufficient information concerning the course of growth in algal cultures. Besides substance production, cell multiplication and chlorophyll formation are important and often fairly independent aspects of the development of a culture. If possible, therefore, one should carry out counts of the cell or colony concentration and determinations of the chlorophyll content in parallel with the photometric determinations.

II. Photometric Determination of Iron Concentration in Synthetic Solutions and Lake Waters.

Introduction.

Owing to its tendency to precipitate from solution after hydrolysis, iron occupies, to a certain degree, a unique position among the constituents necessary for the growth of the algae in cultures. For this reason I shall discuss the source of iron in culture solutions for plankton algae more closely in the next section. The results obtained there are based on iron analyses with o-phenanthroline. In other connections I have tried $\alpha\alpha'$ -dipyridyl for iron determinations in synthetic solutions.

I have also used these iron reagents during recent years for analyses of the iron content in lake waters. Limnologists up to now have, as a rule, used thiocyanate for their iron determinations, unfortunately all too often in an uncritical way. The thiocyanate reaction is thus very sensitive to disturbances by the presence of different ions and colloids, besides which both the amounts of the reagents themselves (thiocyanate and acid) and the time of the reaction have an affect on the resulting colour intensity (see e. g. KOLTHOFF & SANDELL 1943 pp. 670—672, PETERS & FRENCH 1941). Consequently the thiocyanate method has disadvantages and sources of error of various kinds. They could certainly be partly eliminated, if one were completely aware of them, but in the textbooks of hydrochemistry they have not even been indicated, as I have already pointed out in brief (ÅBERG & RODHE 1942 p. 93). The result is that many otherwise careful limnological investigations give useless or directly misleading statements concerning the concentration and condition of iron in natural waters. It is not my intention here to give examples of this unfortunate state of affairs. It would be more useful to point out the new and better possibilities which at present are available for the determination of iron in water.

Directions for the determination of iron with $\alpha\alpha'$ -dipyridyl and with o-phenanthroline.

Since the beginning of the 1930's $\alpha\alpha'$ -dipyridyl and o-phenanthroline have been known as very sensitive reagents for ferrous ions and complexly bound reactive ferrous iron, with which they give an intensive red colour. The first-mentioned compound was used as early as 1933 by MÜLLER for the determination of iron in natural waters, but in limnological circles this has not won the attention which it deserves. Phenanthroline has not yet, to my knowledge, been introduced as a standard reagent for water analyses. The directions given below mainly rest on the experiences of THIEL et al. (1937, 1938) and BOREI (1943) with dipyrldyl, and of FORTUNE & MELLON (1938) with phenanthroline.

A. Iron analysis with $\alpha\alpha'$ -dipyridyl.

1) *Reagents:*

- (a) *Standard acetate — acetic acid buffer:* 500 ml 1 N acetic acid (28.64 ml glacial acetic acid per 500 ml of solution) + 500 ml 1 N Na-acetate (68.04 g Na-acetate $\cdot$ 3 H₂O dissolved in distilled water to 500 ml). pH = 4.6.
- (b) *Sodium sulphite*, fresh 10 % water solution: 10 g Na₂SO₃ are dissolved in distilled water to 100 ml.
- (c) *$\alpha\alpha'$ -dipyridyl*, 0.5 % in an aqueous solution of hydrochloric acid: 0.5 g dipyrldyl is dissolved in 3.32 ml 10 % (2.75 N) HCl and diluted with distilled water to 100 ml (dipyridyl is often obtained in 0.5 g phials; these should not be put in the solution because the glass not seldom contains iron!).

2) *Analytical procedure:*

The size of the sample is adjusted, to the most practicable extent, according to the probable iron content and the layer-thickness with which the photometric or colorimetric determination will be carried out. For a thickness of 10 cm, which was used here, the iron content of the sample should preferably lie between 0.1 and 1.0 mg Fe/l; in other cases the sample would be concentrated or diluted or measured with a more suitable thickness.

50 ml of the water sample (containing if possible between 5 and 50 μ g of iron) are poured into a 100 ml measuring-flask and mixed with 40 ml of reagent (a), 5 ml of reagent (b), 4 ml of reagent (c), and distilled water to 100 ml. The solution is allowed to stand for at least 30 minutes, after which time the colour intensity is constant for several hours.

For the analysis of ferrous iron only, the addition of sulphite is left out (no reduction!).

The result of the analysis is obtained by photometric determination (or, lacking a photometer, by colorimeter). The greatest sensitivity is attained with a blue colour filter (e. g. BG 5 from Schott und Gen., Jena). The galvanometer value (*I*) obtained

for the analysis sample is compared with the galvanometer value (I_0) which is given for the same light intensity with a blank sample (for total iron = sample + reagent (a) + reagent (b) + dist. water to 100 ml; for ferrous iron only = sample + reagent (a) + dist. water to 100 ml). The extinction value is obtained from the quotient by the formula

$$e = \log \frac{I_0}{I}.$$

(The extinction coefficient can be calculated from this by division by the thickness in cms.)

The iron content of the sample is then read from a calibration curve (cf. below), specially prepared for the apparatus in question, giving the relation between the extinction and the iron concentration.

B. Iron analysis with o-phenanthroline.

1) Reagents:

- (a) *Hydroxylamine hydrochloride*, 10 % water solution: 10 g are dissolved in dist. water to 100 ml.
- (b) *o-phenanthroline hydrochloride*, 0.1 % water solution: 0.5 g is dissolved in 500 ml of dist. water.

2) Analytical procedure:

50 ml of the water sample (preferably containing 5—50 μ g of iron) are placed in a 100 ml measuring-flask, together with 1 ml of reagent (a), diluted to about 75 ml with dist. water, and then 5 ml of reagent (b) with dist. water to 100 ml are added. The solution is allowed to stand for at least 30 minutes, after which time the colour reaction reaches a maximum intensity which remains unchanged for several months.

For the analysis of ferrous iron, the addition of hydroxylamine is omitted for both sample and blank sample.

The photometric measurement and calculation of the iron concentration are carried out in the same way as for dipyrldyl.

C. Standard solutions of iron and calibration curves.

It is usually not possible to prepare directly a standard solution with exactly the desired concentration by weighing an iron salt or metallic iron (the textbooks on water analysis give directions which are misleading in this respect). The composition of the iron salts does not generally correspond to the formula given, principally owing to the efflorescence of the salt (if it contains water of crystallization), the transformation of the iron from a bivalent to a trivalent form or vice versa, and to impurities. Even so-called 'pure for analysis' iron wire contains so much impurity that it cannot be used for standard solutions without control measurements.

It is therefore necessary to make quantitative determinations for iron on a stock solution which contains approximately the required amount of iron. These determinations should be preferably both gravimetric and volumetric. Suitable standard solutions are then prepared by diluting this stock solution by appropriate amounts.

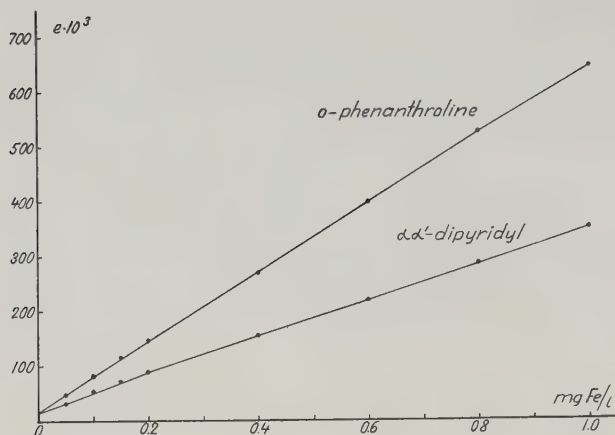


Fig. 6.

Calibration curves for iron determinations with o-phenanthroline and $\alpha\alpha'$ -dipyridyl for colour filter BG 5 and a 10 cm cuvette.

In order to avoid hydrolysis both stock solution and standard solution should be acid, containing, e. g., 4 ml of 10 % HCl per litre solution. One standard solution with 10 mg Fe/l and one with 1.0 mg Fe/l are recommended here, both prepared by suitable dilution of a stock solution with about 1 g Fe/l (the exact concentration of which is consequently determined analytically). For standard solutions of ferric iron, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ is suitable initial material, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ for ferrous standard solutions.

The calibration curve is established by determinations of the extinction values for different Fe-concentrations, obtained by dilution of a standard solution. The calibration curves for dipyrldyl and phenanthroline given in fig. 6 were obtained from a standard ferric solution with 10 mg Fe/l. These curves, which are only strictly valid for the given conditions of determination (colour filter BG 5, thickness 10 cm and the photocell in question), show that the phenanthroline reaction is about twice as sensitive as the dipyrldyl reaction and that LAMBERT-BEER'S Law (extinction is a linear function of concentration) holds at least to a concentration of 1 mg Fe/l (at 1.5—1.7 mg Fe/l, the slope of the curve for phenanthroline begins to decrease markedly). It can be pointed out here that the thiocyanate reaction is somewhat less sensitive than the dipyrldyl reaction.

Determinations of iron concentration in synthetic solutions with dipyrldyl and with phenanthroline.

In order to gain an idea of the accuracy of the iron determinations the following experiment was made:

Solution A: 1.0 mg ferric iron/l in acid solution was obtained by dilution of a ferric stock solution with an iron content established both gravimetrically and volumetrically.

Solution B: About 1 mg of ferrous iron/l was obtained by dissolving 0.2489 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 500 ml of dist. water, after which 10 ml of it were diluted to 1,000 ml.

Solution C: Was obtained by mixing equal parts of solution A and solution B.

1) *Determination of Fe-concentration in different dilutions of solution B.*

Analyses were carried out on:

- (a) 50 ml B + 0 ml dist. water,
- (b) 30 ml B + 20 ml dist. water,
- (c) 10 ml B + 40 ml dist. water.

The results, given in mg per litre of solution B, appear in Table 2.

Table 2. Results of iron analyses on different dilutions of solution B (ferrous sulphate), calculated in mg per litre of this solution.

Dilution	Phenanthroline			Dipyridyl		
	Fe ⁺⁺	Fe ⁺⁺⁺	total	Fe ⁺⁺	Fe ⁺⁺⁺	total
(a)	0.88	0.01	0.89	0.85	0.02	0.87
(b)	0.82	0.03	0.85	0.86	0.04	0.90
(c)	0.80	0.06	0.86	0.75	0.15	0.90
Average			0.87			0.89

2) *Determination of Fe-concentration in different dilutions of solution C.*

Analyses were made on:

- (a) 50 ml C + 0 ml dist. water,
- (b) 30 ml C + 20 ml dist. water,
- (c) 10 ml C + 40 ml dist. water.

The results, given in mg per litre of solution C, appear in Table 3.

Table 3. Results of iron analyses on different dilutions of solution C (mixture of ferric chloride and ferrous sulphate), calculated in mg per litre of this solution.

Dilution	Phenanthroline			Dipyridyl		
	Fe ⁺⁺	Fe ⁺⁺⁺	total	Fe ⁺⁺	Fe ⁺⁺⁺	total
(a)	0.44	0.50	0.94	0.41	0.50	0.91
(b)	0.41	0.52	0.93	—	—	—
(c)	0.40	0.53	0.93	0.36	0.57	0.93
Average			0.93			0.92

On the basis of the values for the total iron content, we can establish that the limits of error for each method of determination, as between the two methods themselves, amount to about $\pm 2\%$; a not inconsiderable part of the deviations must, however, be ascribed to errors in using the pipettes. The certainty of the methods seems to be about the same if the analysis sample contains about $9\text{ }\mu\text{g Fe}$, dilution (c), or $45\text{ }\mu\text{g}$, dilution (a).

From the separate values for ferric and ferrous iron we find further that increased dilution resulted in a decrease of the ferrous content and an increase of the ferric concentration. This relation, which is dependent on the oxidation of bivalent ions to trivalent ions by the oxygen dissolved in the water, must be paid attention to in the use of standard ferrous solutions.

Finally, with the help of the values of the total amount of iron given in Table 2, we can confirm the statement made above (p. 25) that the chemical formula of an iron salt is not applicable for an exact forecast of the Fe-concentration in a solution containing a weighed amount. $0.2489\text{ g FeSO}_4 \cdot 7\text{ H}_2\text{O}$ should contain, according to the formula, 50 mg Fe , wherefore solution B should have a concentration of 1.0 mg Fe/l . However, the average value for both the methods was 0.88 mg Fe/l .

The solutions analysed in the experiment described here represent very uncomplicated conditions, where the iron exists completely in the ionic form. In the next section I shall describe results of analyses of solutions containing ionic or compounded iron as well as colloidal and precipitated ferric hydroxide. In the latter forms the iron is not recorded by direct analysis with dipyrldyl or phenanthroline. This may appear to be a weakness but from a biological viewpoint it is an advantage, because the reaction between iron and dipyrldyl or phenanthroline records the concentration of the most easily assimilable iron. If, however, one wishes to determine the total iron content of a solution which contains compounded or colloidal iron, this can easily be done by evaporating the sample and igniting the residue. This is redissolved in acid solution and the analysis is then made on this solution.

The dipyrldyl and the phenanthroline methods are decidedly superior to the thiocyanate method, concerning both sensibility and reliability. In the choice between dipyrldyl and phenanthroline I recommend the latter reagent. On one hand the phenanthroline reaction is nearly twice as strong in colour as the dipyrldyl reaction and on the other hand the analytical procedure is considerably simpler for phenanthroline than for dipyrldyl. This latter depends on the fact that the pH limits of the maximum colour reaction are considerably wider for phenanthroline (the pH of the sample on the addition of the phenanthroline should lie between 2 and 9) than

for dipyridyl (pH 3.5—8.5, but the sulphite further narrows the interval, because complete reduction requires acid reaction). In addition, hydroxylamine, which is a very convenient reducing agent, cannot be used for dipyridyl. Finally, the consumption of reagents is greater and more costly for the dipyridyl method.

Determinations of iron concentration in lake waters with thiocyanate and with phenanthroline.

From the ecological viewpoint it is a very great disadvantage that, in the determination of iron in natural waters by the thiocyanate method, a direct analysis does not give definite results in the presence of humus or other organic substances. In the numerous cases in the limnological literature where such direct determinations were carried out, the results must at least be regarded as uncertain; if the water in question was rich in organic substances or contained humus, the analysis results are without doubt useless (as a rule too high).

With the thiocyanate method one can, in general, only after oxidizing and evaporating the water sample (and possibly also igniting the residue) determine the iron content, but only the total value of this. The biologically important concentration of reactive iron on the other hand is not available for analysis with this method.

It should therefore be of extraordinarily great value if the phenanthroline reaction, on the ground of its insensibility to changes of pH within the range for natural waters and owing to its certainty even in the presence of other ions or colloids, could be used directly in all cases where the water is not so strongly coloured by humus or made cloudy by colloids that the photometric measurement is made difficult or prevented by these causes.

So far I have not had opportunity to make more than a few isolated measurements of the active iron content of lake waters by phenanthroline. The results appear, however, to be of so great interest that two of them deserve to be quoted. These have been obtained for waters from the eutrophic lake Erken (70 km east of Uppsala) on the one hand, and from the humic-oligotrophic Siggeforasjön (Siggefora Lake, 45 km north-west of Uppsala) on the other. For comparison a few earlier values of the total iron content are stated in Table 4. They are obtained by the thiocyanate method after ignition and redissolution (with hydrochloric acid) of the residue obtained from evaporation of 500 ml surface water.

Table 4. The total iron concentration (mg/l) of surface water according to measurements by the thiocyanate method.

E r k e n		S i g g e f o r a s j ö n	
Date	mg Fe/l	Date	mg Fe/l
28.V. 1941	0.16	26.V. 1941	0.70
11.VII. 1941	0.09	7.VII. 1941	0.50
14.VIII. 1941	0.14	23.VIII. 1941	0.64
27.X. 1941	0.10	29.X. 1941	0.26

In agreement with the general experience that the total iron content to a large extent goes parallel with the humus content (= water colour; cf. ÅBERG & RODHE 1942 p. 168), the total concentration of iron in Siggeforasjön is considerably higher than in Erken. One should be able to expect that approximately the same holds for the concentration of the reactive iron.

Direct phenanthroline analyses of newly taken samples of lake water gave, however, the surprising result that the amount of reactive iron not only in Erken but also in Siggeforasjön was so unimportant that the scarcely discernible red colouring was immeasurable. The water samples were therefore concentrated on a waterbath (from 500 ml to 50 ml; for Erken a parallel sample was evaporated to dryness and the residue was dissolved in water without addition of acid). The results given in Table 5 were then obtained by the phenanthroline method.

Table 5.¹ The concentration of the reactive iron (mg/l) according to measurements by the phenanthroline method after concentration of the water samples (the values given in brackets refer to parallel samples which were completely evaporated and then redissolved in water).

E r k e n 19.V. 1944		S i g g e f o r a s j ö n 13.VI. 1944	
0 m	0.01 (0.03)	0 m	0.03
18 m	0.01 (0.02)	10 m	0.05

These first determinations with phenanthroline which, in the case of Erken, I have confirmed by some further analyses on later occasions, show that it is only a small part of the total iron content which reacts directly (after reduction) with phenanthroline. The main part of the iron is clearly firmly bound in organic compounds or exists in a colloidal and a precipitated

state as ferric hydroxide or iron-humus-complexes. HUTCHINSON (1941 p. 53) obtained analogous results for the surface water of Linsley Pond (Connecticut) where some analyses with dipyrityl gave an average of 0.175 mg total iron per litre; of this only 0.035 mg/l was in solution (of which 0.030 mg/l was in organic form) while the rest was found to be sestonic (0.075 mg/l being organically combined).

According to the values given above in Tables 4 and 5, the concentration of the reactive iron appears to be independent of the total iron content. Therefore the measurements of the latter cannot give any information on the amounts of iron at the disposal of the organisms. Thus, the surface water of Siggeforasjön had about 0.40 mg/l more total iron than had the surface water of Erken, but its concentration of reactive iron was only about 0.02 mg/l higher than in Erken.

The non-reactive iron is certainly inactive in different ways and to different degrees. The values given in brackets in Table 5 show that even an evaporation to dryness (without the addition of chemicals) liberates a part of it. Many important problems in this field call for continued investigations.

In a later connection (p. 114) I shall show that the content of reactive iron of lake water (measured by o-phenanthroline) seems to agree with the concentration of assimilable iron (measured biologically).

III. The Source of Iron in Nutrient Solutions.

Introduction.

In my first experiments to cultivate plankton algae it proved to be very difficult or often absolutely impossible to obtain satisfactory growth in autoclaved culture solutions containing only inorganic salts. On the other hand considerably better yields were obtained if the solutions were not autoclaved before inoculation. But in that case other organisms (bacteria, fungi, and especially algae of the *Chlorella* type) also began to grow in the cultures, whereby an exact study of the inoculated alga was made impossible.

However, even after autoclaving, good growth could often be attained if a few ml of soil extract were added to each litre of culture solution before sterilization. This confirmed the experiences of PRINGSHEIM (1936), who found that the addition of soil extract or of humus preparations etc. was favourable, or necessary, for the growth of several algae in pure cultures.

PRINGSHEIM could not definitely solve "das Rätsel der Erdabkochung" but appears most nearly to tend to the opinion that the active principle of the soil extract in one way or other can often be connected with the maintenance of iron of the algae. This assumption is further emphasized by him in his guide to the methods of algal cultures (1946 p. 42), where he nevertheless points out that "it is not fully known what properties are responsible for the beneficial influence of soil extract."

In this connection there exists no occasion to enter more closely upon the complicated question concerning the causes for the stimulating effect of soil extract on growth. This effect is, even according to my experiences, of a composite character and it can therefore have different significances for different algae. The positive effect is often without doubt completely bound up with the iron factor, because for many plankton algae (especially Chlorococcales and diatoms) I have, by suitable choice of the iron source, been successful in excluding the addition of soil extract without the growth or final yield being thereby impaired. In the following section an account is given of the experiments which resulted in an improved source of iron for culture solutions.

Ferric chloride and ferric citrate.

In order to establish the effect of acidification and the effect of soil extract on the stability of the dissolved iron before and after the autoclaving of the solution, the following experiment was carried out. As stock solution was used on the one hand (a) a newly prepared FeCl_3 solution containing 2.7 mg Fe/l, and on the other, (b) and (c), a portion of this solution which was mixed with 4 ml of 10 % HCl per litre. Three solutions were prepared by dilution:

- (a) with 0.27 mg Fe/l. Neutral FeCl_3 solution as iron source.
- (b) with 0.27 mg Fe/l. Acid FeCl_3 solution as iron source.
- (c) as (b), but with the addition of 10 parts of soil extract to 100 parts of solution.

Photometric determinations of iron with o-phenanthroline gave the following results:

	(a)	(b)	(c)
before autoclaving:	0.15	0.27	0.30 mg Fe/l,
after autoclaving:	0.005	0.005	0.30 mg Fe/l.

Consequently by using a neutral ferric chloride solution, nearly one half of the iron originally added had been transferred to a non-reactive form even before autoclaving. No precipitate could be observed, but the brown colour of the solution showed that the ferric chloride had been partly hydrolysed to colloidal ferric hydroxide. By autoclaving, the hydrolysis was speeded up, so that practically all the iron, be it added from neutral or acid ferric chloride solution, was quickly transferred to ferric hydroxide, which now was precipitated as a flocculent precipitate.

On the other hand the hydrolysis failed to appear, even after autoclaving, in the presence of soil extract, which obviously itself contained iron in reactive form. This effect must be assumed to depend on the complex combination of the iron with organic compounds in the soil extract (PRINGSHEIM 1934 p. 302).

It has long been known that inorganic iron salts, on the basis of their tendency to hydrolyse in neutral or (especially) alkaline reaction, are not suitable as iron sources in culture solutions. HOPKINS & WANN (1927), for this reason, suggested the addition of sodium citrate to the culture solution so that the iron, which was added as ferric chloride, might be kept in solution in a complex form. Later a number of workers have preferred to add the iron directly as ferric citrate or ferric ammonium citrate, whereby they often obtained, with different objects, good growth in synthetic so-

lutions. CHU (1942), on the other hand, has used ferric chloride only as iron source for algal culture media, which I have found to be a weakness in his otherwise often well-composed solutions (cf. pp. 45, 60).

Even from the preliminary experiments I could establish that a culture solution with iron added as ferric citrate offers, after autoclaving, better conditions for growth by, e. g., *Ankistrodesmus falcatus*, *Eudorina elegans*, or *Asterionella formosa* than if the iron were added as ferric chloride. It was, however, also obvious that ferric citrate is not an ideal source of iron either; it is thus not stable in aqueous solution, particularly not in light. A newly prepared ferric citrate solution, containing e. g. 0.1 mg ferric citrate per ml, has a yellow colour, but in light even after a few days it begins to develop a brownish colour. This asserts itself all the more until the brown solution, after about two weeks, rapidly loses its colour, owing to the iron being practically completely precipitated as a flocculent brown precipitate of ferric hydroxide.

The instability of ferric citrate increases with increasing temperature. Autoclaving has therefore a fatal effect on the concentration of reactive iron, as the following analysis values show. Half of a newly prepared ferric citrate solution containing 17.5 mg Fe/l was immediately autoclaved. After three days in light this half was considerably browner than the non-autoclaved portion and iron analysis with phenanthroline then gave the following result:

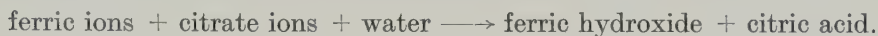
non-autoclaved solution:	8.6 mg Fe/l,
autoclaved solution:	0.6 mg Fe/l.

Thus it is seen that iron added as ferric citrate is unstable in light and that the diminution of the reactive iron concentration is accelerated to a high degree by an autoclaving. It should be especially emphasized that no visible flocculence had yet taken place when these analyses were made. The iron was consequently in solution the whole time (no residue was obtained on filtration), but after hydrolysis it had apparently gone over to colloidal form. In this form the iron does not react with o-phenanthroline.

This instability of ferric citrate in light must, for algal cultures which demand access to light, be directly disadvantageous, particularly for quantitative experiments, where it is intended to keep the separate factors under control. An exact establishment of the iron needs of an alga is of course impossible, if the iron cannot be supplied in a reliable way. It is furthermore impracticable, when synthesizing culture solutions, to be obliged to work with an unstable stock solution for iron, which must often be newly prepared.

Ferric citrate in combination with citric acid.

Fortunately it proved to be not so difficult to bring about a stabilization of ferric citrate in aqueous solution. I proceeded from the assumption that the course of the reaction in an unstable ferric citrate solution should, in principle, be the following:



If this process is reversible, its tendency according to the scheme to go to the right should be able to be counteracted by the addition of citric acid. By such an addition one should also be able to facilitate the binding of the iron in complex ions, besides which the accompanying lowering of the pH can be assumed to be advantageous from different points of view.

On the basis of this line of thought the following experiment was made on the amounts of reactive iron in solutions of ferric citrate (A) and ferric ammonium citrate (B), with (A 2 and B 2) and without (A 1 and B 1) the addition of citric acid:

- A 1: 25 mg *ferric citrate* in 250 ml dist. water, brought into solution by being stored overnight at 60° C. Contains 100 mg ferric citrate per litre (= 18.7 mg ferric iron according to the molecular weight 299 with one atom Fe). Colour at the beginning slightly yellow, later brownish.
- A 2: 20 mg *ferric citrate* + 20 mg *citric acid* dissolved as above in 200 ml dist. water. Contains 100 mg ferric citrate and 100 mg citric acid per litre. Colour slightly greenish.
- B 1: 10 mg *ferric ammonium citrate* (powdered) in 100 ml dist. water. Dissolves immediately with a brown colour. Contains 100 mg ferric ammonium citrate per litre (Fe content cannot be calculated, since the composition changes from preparation to preparation).
- B 2: 5 mg *citric acid* added to 50 ml B 1. Contains 100 mg ferric ammonium citrate and 100 mg citric acid per litre. The original brown colour of the solution quickly fades and in a few hours changes to slightly greenish.

The solutions were placed (without previous autoclaving) in diffuse daylight. 12 hours, 3 days, 5 days, and 30 days after the start of the experiment, 2 × 0.5 ml of solution were taken out of each flask for analysis with phenanthroline for ferrous and ferrous + ferric iron. The results, given as mg Fe/l, are seen in Table 6.

These analysis values indicate for ferric citrate that the addition of equal amounts by weight of citric acid stabilizes the reactive iron, which otherwise rapidly decreases in light, finally precipitating as ferric hydroxide. Concerning ferric ammonium citrate, the values show that even in a newly prepared solution only a rather small part of the iron exists in the reactive form; judging from the initially brown colour of the solution, the

Table 6. The concentration of reactive iron (mg/l) in solutions of ferric citrate (A) and ferric ammonium citrate (B), in the absence (A 1 and B 1) and presence (A 2 and B 2) of citric acid. The solutions placed in diffuse daylight.

		12 hours	3 days	5 days	30 days	
A 1	ferrous	3.7	6.7	6.0	0.0	precipitation
	ferrous + ferric	17.3	12.2	9.6	0.3	
A 2	ferrous	3.3	9.5	9.9	10.3	green
	ferrous + ferric	17.3	16.9	16.9	17.5	
B 1	ferrous	0.4	0.0	0.0	0.0	brown
	ferrous + ferric	8.7	5.0	2.0	0.0	
B 2	ferrous	4.8	17.1	20.1	18.8	green
	ferrous + ferric	15.0	28.9	29.9	29.7	

rest of the iron exists as colloidal ferric hydroxide. This is obviously considerably more stable in the ferric ammonium citrate solution (B 1) than in the ferric citrate solution (A 1), because the content of reactive iron in the former solution goes down to zero without any flocculent precipitation taking place. The addition of citric acid in the proportion 1 : 1 changes the iron of the ferric ammonium citrate to a reactive form, the colour of the solution going from brown to green; probably a complex combination between the iron and the citrate ions takes place here. We notice that this process is not momentary but is going well 12 hours after the preparation of the solution (B 2), and that even after three days it is not completed (28.9 mg reactive iron per litre); five days after the start of the experiment, however, the same concentration level is recorded (29.9—29.7 mg Fe/l) as after 30 days.

In order to get an idea of the intensity of the brown colour in solution B 1 a colorimetric comparison was made on the third day of the experiment between this solution and K_2PtCl_6 -standard (ÅBERG & RODHE 1942 p. 87). 2 ml of B 1 were diluted to 50 ml and poured into a colorimeter tube; in order to obtain the same colour in another tube 1.0 ml of standard solution had to be added to 49 ml of dist. water. Consequently the colour in solution B 1 corresponds to $1.0 \times 20 \times 25 = 500$ mg Pt/l. If this colour is wholly due to colloidal ferric hydroxide, the concentration of the iron existing in this form should be of the order of magnitude of $500/20 = 25$ mg Fe/l, according to ÅBERG & RODHE (loc. cit., p. 142). This agrees well with the analysis values in Table 6, according to which the total iron content in the ferric ammonium citrate solution is about 30 mg/l, of which on the third day of the experiment 5.0 mg/l was in a reactive form in B 1.

The remainder, 25 mg/l, I assumed above to exist as ferric hydroxide sol, an assumption which is consequently confirmed by the colorimetric measurement.

It further follows from the values in Table 6 that the reactive iron from the beginning is to a rather large extent in the trivalent form. An increasing part of it changes to ferrous iron owing to the effect of the citric acid, and after one month 60—65 % of the iron exists in the bivalent form (A 2 and B 2). This reduction of ferric iron must imply an oxidative decomposition of the citric acid, i. e., one can expect that the stability of the iron in a solution with ferric citrate and citric acid is dependent on the amount of citric acid. In order to investigate this the following experiment was carried out.

Solution A: 0.1 mg *ferric citrate* per ml (50 mg dissolved at 60° C in 500 ml dist. water).

Solution B: 10 mg *citric acid* per ml (1.0 g dissolved in dist. water to 100 ml).

The following mixtures were obtained by adding different amounts of solution B to 50 ml A (= 5 mg ferric citrate):

Solution	ml B	ml dist. water	Citric acid	pH
			Ferric citrate	
(a)	0.0	5.0	0	3.43
(b)	0.5	4.5	1	3.10
(c)	1.0	4.0	2	2.96
(d)	1.5	3.5	3	2.89
(e)	2.0	3.0	4	2.81
(f)	2.5	2.5	5	2.75
(g)	5.0	0.0	10	2.58

The colour of the newly prepared solutions increased continuously from slightly light green for solution (a) to rather strong green for solution (g). Their pH values, as measured with a glass electrode, are given above. They were poured into 100 ml conical flasks, supplied with cotton wool stoppers, autoclaved, and afterwards kept at room temperatures in diffuse daylight. For each iron analysis 0.5 ml was taken out and diluted to 50 ml, mixed with hydroxylamine hydrochloride and phenanthroline, and measured photometrically. The analysis values, given in mg Fe per litre of the original solutions (a)—(g), are presented in graphical form in fig. 7.

It is obvious that the stability of the iron in a ferric citrate solution increases with the addition of citric acid. With the aid of fig. 7 we could (partly by extrapolation) establish that the iron completely disappeared from the solutions (by precipitation as ferric hydroxide) after approximately the following times:

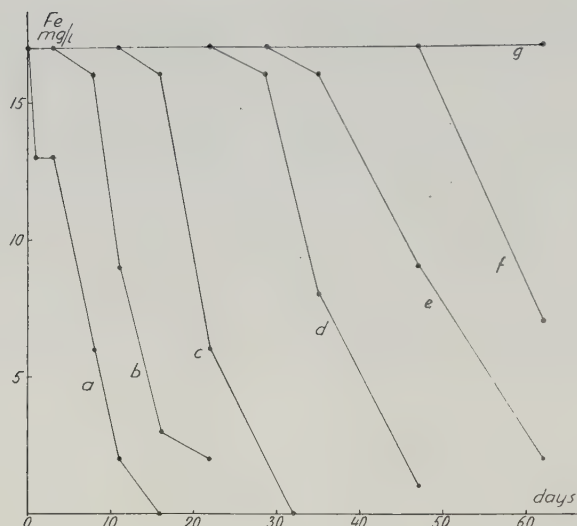


Fig. 7.

Decrease of reactive iron with time in solutions of ferric citrate for varying amounts of citric acid. Refer to the text.

Solution	mg citric acid per mg ferric citrate	Number of days until Fe = 0 mg/l	Extension of time (days) by the citric acid
(a)	0	16	
(b)	1	25	9
(c)	2	32	16
(d)	3	49	33
(e)	4	66	50
(f)	5	75	59
(g)	10	> 75	> 59

Judging from the last column, the time of stability is not a function of the amount of citric acid only, since the extension of this time is apparently greater than the corresponding relative increase in the additions of citric acid. Since these diminish the pH of the solution one can assume that it is at the same time a question of a pH effect. This assumption is confirmed by the fact that more concentrated solutions, where the citric acid is able to depress the pH to a greater extent, are stabilized for a very long time, even in light, with only one part citric acid to one part ferric citrate. The complete course of events becomes fairly clear if we make use of some of the available information in the literature on this subject.

PELTZ & LYNN (1938) found that ferric salts and ferric hydroxide in light oxidize citric acid (giving rise to free carbon dioxide) and that the iron is thereby reduced to a soluble ferrous salt, probably a citrate. However the ferrous iron is easily reoxidized to trivalent iron by the oxygen of the solution and that absorbed from the air. According to BRYAN (1933) this oxidation is greatly pH-dependent and is considerably accelerated by an increase of pH from, e. g., 3.3 to 5.7. Also below pH 3.3 an acidification delays oxidation, as well as decreasing the velocity with which a ferrous citrate solution absorbs atmospheric oxygen (BRYAN 1933 p. 831). The effect of the citric acid on the ferric citrate and ferric ammonium citrate in aqueous solution can therefore be assumed to be threefold: partly, the citric acid facilitates the complex combination of the iron with citrate ions, whereby the iron is stabilized in the reactive form, partly, it serves as a reducing agent, whereby a part of the ferric iron is converted to ferrous iron, and partly, it diminishes the pH of the solution, whereby the oxidation of the ferrous iron is delayed. When the citric acid is consumed, the iron-citrate complex is obviously not sufficiently stable to prevent a hydrolysis to ferric hydroxide, which quickly and completely precipitates, provided it is not held in solution as a stable sol (cf. the ferric ammonium citrate in Table 6).

Ferric citrate and ferric chloride in combination with citrates.

Potassium or sodium citrate, according to PELTZ & LYNN, have not the same effect on ferric hydroxide as citric acid has. They do not reduce the iron and do not therefore decompose even in bright sunlight. They are, however, in this connection, of interest, since HOPKINS & WANN (1927) and HOPKINS (1930) and other workers have used additions of citrates to hold iron in solution in culture media for algae. In order to compare directly the effect of the citric acid and the citrates on the stability of the iron in a ferric citrate solution I have carried out the following experiment.

Solutions:

- I. 50 mg *ferric citrate* dissolved at 60° C in 500 ml dist. water.
- II. 1.0 g *citric acid* dissolved in 100 ml dist. water.
- III. 1.0 g *potassium citrate* dissolved in 100 ml dist. water.
- IV. 1.0 g *sodium citrate* dissolved in 100 ml dist. water.

From these the following mixtures were made in 100 ml conical flasks:

- 1) 50 ml I + 0.5 ml II + 2.0 ml dist. water. In light.
- 2) 50 » I + 0.5 » II + 2.0 » » » In darkness.
- 3) 50 ml I + 0.5 ml II + 2.0 ml dist. water. In light, with superstratum of liquid paraffin.

4) 50	»	I + 0.0	»	III + 2.5	»	»	»	} In diffuse daylight.
5) 50	»	I + 0.5	»	III + 2.0	»	»	»	
6) 50	»	I + 1.5	»	III + 1.0	»	»	»	
7) 50	»	I + 2.5	»	III + 0.0	»	»	»	
8) 50	»	I + 0.5	»	IV + 2.0	»	»	»	
9) 50	»	I + 1.5	»	IV + 1.0	»	»	»	
10) 50	»	I + 2.5	»	IV + 0.0	»	»	»	

All the flasks were provided with cotton wool stoppers and autoclaved. Flask 3 was then provided with a layer of liquid paraffin and a rubber stopper to prevent the access of air to the solution. Iron analyses with phenanthroline gave the results shown in Table 7.

Table 7. The concentration of reactive iron (mg/l) in a solution of ferric citrate mixed with citric acid (1—3), potassium citrate (5—7), or sodium citrate (8—10).

Flask	Start (before autoclaving)		After four days		After five months	
	ferrous	total	ferrous	total	solution	precipitation
1	5.6	15.7	9.1	16.5	colourless	brown
2			5.5	16.5	green	none
3			8.8	16.4	colourless	brown
4			1.3	6.3	} colourless	} brown
5	0.8	15.7	0.0	0.9		
6			0.0	0.7		
7			0.0	1.0		
8	0.8	15.6	0.0	0.6		
9			0.0	0.5		
10			0.0	0.3		

It follows from the results for flasks 1—3 that a ferric citrate solution placed in the dark, containing an equal amount by weight of citric acid, is stable for at least five months, whilst that placed in the light is unstable, even if access to air is prevented.

The analysis values for flasks 4—10 show that neither potassium citrate nor sodium citrate increases the stability of the ferric citrate, rather, they work in the opposite direction. Neither have they any reducing effect, since in their presence ferrous iron is completely lacking. After four days the concentration of reactive iron had decreased considerably more in the flasks with the addition of potassium or sodium citrate than in the flask (No. 4) which contained ferric citrate only. Their colour was more or less

brown, but no precipitation could be observed. It appears probable therefore that the K- and Na-citrate accelerated the hydrolysis (they have slightly alkaline reaction) to colloidal ferric hydroxide. Whilst that in flask 4 precipitated within a month, the solutions with added citrate kept for a longer time (but less than five months). A peptizing effect of sodium and potassium citrate seems to be probable.

In order to investigate the effect of potassium citrate on the stability of iron in a ferric chloride solution, the source of iron which HOPKINS & WANN and USPENSKI 'buffered' in this way, I prepared a solution containing 1.0 mg FeCl_3 and 2.0 mg K-citrate per ml, i. e., equimolar amounts of the two salts. The total concentration of iron of the solution was 344 mg/l. Its colour at the beginning was slightly yellow, but after some time in diffuse daylight this darkened and after six weeks it was pure brown; subsequently the colour appeared to be constant. Seven weeks after the preparation of the solution, analyses were made with phenanthroline. Ferrous iron was completely lacking and the concentration of the reactive ferric iron was only 110 mg/l. About $\frac{2}{3}$ of the total iron content of the solution was consequently inaccessible to direct analysis and this part of the iron must be considered to exist as stable ferric hydroxide sol. In a parallel solution with ferric chloride only, hydrolysis had, on the other hand, taken place and all the iron content had precipitated to the bottom. The potassium citrate had clearly prevented this precipitation by peptizing the ferric hydroxide, but this stabilization at the same time involved an inactivation of the iron.

HOPKINS (1930) has made measurements of the depression of the freezing point and the conductivity in different mixtures of ferric chloride and potassium and sodium citrate respectively. He found that for a certain proportion of both the components there was a sharp break in the curve, and tried to furnish proof of a complex formation taking place at this point. Thus he started from the fact that ferric hydroxide is practically insoluble, but did not notice that under suitable conditions it can remain in solution as a very permanent sol. The break in the curve can equally well be interpreted as the most advantageous combination for the peptization of the ferric hydroxide. Such an interpretation is in keeping with the results obtained by ZAIDES (1935) according to which "the interaction of $\text{Fe}(\text{OH})_3$ with HCOOH and AcOH results only in peptization and no formation of chemical compounds. Ferric salts with Na-formate and acetate in equivalent amounts form colloidal solutions. Thus, contrary to the literature data, Fe-salts with Na-salts of organic acids form no cation complexes of a constant composition" (according to Chemical Abstracts 30: 2173. 1936). On the other hand he found that anion complexes of molecular degree of

dispersion could be formed, when e. g. Na-oxalate was mixed in solution with a ferric salt; at the same time, however, the mentioned peptization of ferric hydroxide took place.

This standpoint gives another meaning to the 'buffering' with citrate than that conceived by USPENSKI & USPENSKAJA (1925), USPENSKI (1927), HOPKINS & WANN (1927), HOPKINS (1930), and PRINGSHEIM (1934), when they stabilized the iron content of the culture solutions by the additions of citrate. Such a stabilization does not seem, as these authors assumed, to depend on the entry of iron into organic ion or molecular complexes, but it rather appears to be a peptization of the ferric hydroxide formed after hydrolysis, so that this can be held in solution in the colloidal form (cf. HARVEY 1937 p. 216). The increase of the size of the particles (from ions or molecules to colloids) is, at the same time, to a greater or lesser extent, accompanied by an inactivation of the iron from the chemical point of view and perhaps from the biological also. A stabilization of the iron in this way can scarcely be appropriate for culture solutions, where definite conditions are desired.

The application of the experimental results to culture solutions.

The experiments mentioned have given the result that, as the source of iron in culture solutions, it should be possible to use with advantage ferric citrate in combination with citric acid. By the addition of citric acid the ferric citrate solution is stabilized without the iron being inactivated.

In order to avoid a too large addition of an organic substance (a source of carbon!) I have chosen the proportion 1 part of citric acid to 1 part of ferric citrate. A stock solution with this composition can be autoclaved many times and can be stored in the dark for an almost unlimited time.

In ordinary culture solutions, the pH value lies about or above the neutral point. The stabilizing effect of the citric acid must, for this reason, be considerably less in the culture solution itself than in the stock solution. This appears from the following analysis values obtained before and after the autoclaving of a culture solution, to 1 litre of which was added 0.57 ml of 'ferric citrate — citric acid solution 10/1 + 10/1' (= 10 mg ferric citrate and 10 mg citric acid per ml stock solution), an addition calculated to give 1.0 mg Fe per litre of culture solution:

before autoclaving: 1.02 mg Fe/l,
after autoclaving: 0.53 mg Fe/l.

In order to avoid this change of the concentration of the reactive iron on autoclaving culture solutions, the Fe stock solution should be autoclaved separately and additions from it should be made after the sterilization of the culture medium. This addition should first be done when the culture solution is to be used.

Against the use of the proportion 1 part citric acid to 1 part ferric citrate, it may be objected that the stabilizing effect is of rather short duration (about one week). If so wished the effect can be extended by increasing the amount of citric acid (cf. fig. 7). In my experiments I have, however, obtained completely satisfactory results (even in quantitative series with different amounts of iron) using a stock solution containing, e. g., 0.1 mg ferric citrate and 0.1 mg citric acid per ml (cf. pp. 50 and 105).

IV. A Nutrient Solution for Autotrophic Plankton Algae.

Introduction.

On the basis of experiences from nature it must be considered to be a fact beyond dispute that different plankton algae can have widely differing environmental requirements. Thus many species prefer eutrophic waters, while others only or mainly occur in waters of a completely different character. After establishing this fact, the next task is to explain it. However, this is not as a rule possible with the help of observations from nature alone. In order to penetrate those complex connections which are in question here, one must be able to vary one factor at a time, the others being constant. Such a task can generally not be answered, as far as plankton algae are concerned, without access to pure cultures under controlled conditions.

Even if the requirements are more or less different, they are apparently so nearly in agreement for many plankton algae, that one and the same water provides accommodation for quite a number — often several hundred — of species at the same time. In an analogous way — so it seems — one could make up a synthetic environment, in which not only one but perhaps most of the plankton algae occurring simultaneously in a lake could be cultivated. Such a culture solution obviously cannot offer optimum conditions for all these algae, but it should be able to give a starting point for continued experiments on the minimum-optimum-maximum positions of the various factors for the different species. Even for the study of algae which do not allow themselves to be cultivated in this culture solution under suitable conditions of light and temperature, the medium could be of value, because it would facilitate the tracing of the factors on which these divergent algae make particular demands (“more” or “less” than in the culture solution). This line of thought is the basis of the following experiments.

Experiments with *Ankistrodesmus falcatus*.

The test object here has been *Ankistrodesmus falcatus* (CORDA) RALFS in bacteria-free pure culture (clone), originating from the lake Erken,

where the alga was taken in a net plankton sample on November 24th 1943. A few drops of the sample were added the same day to a culture solution according to CHU No. 10 (CHU 1942 p. 298) containing 10 % soil extract (prepared according to PRINGSHEIM 1936), which was placed in a photothermostat at 20° C. Here *A. falcatus* grew fairly well together with some other plankton algae, especially Chlorococcales, and the rough culture was reinoculated a few times into the same nutrient solution. On January 9th 1944 three *Ankistrodesmus* cells were isolated, with a micromanipulator and under sterile conditions, each into a culture tube with 10 ml of autoclaved culture solution Chu 10 + 10 % soil extract. After three weeks a fine growth was obtained in two of the tubes. Usual tests (both aerobic and anaerobic) on peptone agar proved the cultures to be free from bacteria.

In Chu 10 without soil extract the growth of *Ankistrodesmus falcatus* (as of all other investigated algae) was considerably inferior to that in the culture solution containing such an extract. Since I learnt to use ferric citrate — citric acid as iron source, relatively good growth was obtained in the following completely synthetic medium, called culture solution V:

Culture solution V.

	mg/l		mg/l	mg/ml in stock solu- tion	ml of stock solution per litre medium
Ca	4.0	Ca(HCO ₃) ₂	16.0	solid	10 mg CaCO ₃ ¹
Mg	5.0	MgSO ₄	25.0	1	25
Na	7.5	Na ₂ SiO ₃	20.0	1	20
K	2.2	K ₂ HPO ₄	5.0	1	5
Cl	13.3	NH ₄ Cl	20.0	1	20
SO ₄	20.0	MgSO ₄	25.0		
HCO ₃	12.0	Ca(HCO ₃) ₂	16.0		
N	5.25	NH ₄ Cl	20.0		
P	0.89	K ₂ HPO ₄	5.0		
Fe	0.18	Ferric citrate — citric acid	1.0 + 1.0	0.1 + 0.1	10 ²
Mn	0.01	MnSO ₄	0.03	0.1	0.3
Si	4.6	Na ₂ SiO ₃	20.0		

¹ The dissolution of CaCO₃ can be accelerated by bubbling through CO₂.

² The iron stock solution is autoclaved separately and is added to the sterile culture solution. It must be kept in the dark.

The continued experiments with the cultivation of different algae in culture solution V were however not satisfying. Diatoms (*Asterionella for-*

mosa, *Melosira islandica* subsp. *helvetica*, and others) grew at the most for a short time but then they died away, if reinoculation was not carried out. Green algae (species of the genera *Ankistrodesmus*, *Scenedesmus*, *Coelastrum*, *Pediastrum*, as well as *Eudorina elegans* and *Pandorina morum* etc.) seemed to thrive better, but after only 2 to 3 weeks even the best cultures (and in the first place just these) began to turn yellow, the algae often adhering to the walls of the culture vessel. Culture solution V was clearly unsuitable for them from a long term point of view. In order to proceed to a better medium I performed therefore, with *Ankistrodesmus falcatus* as test object, a series of tests, in which each one of the salts present in culture solution V was added stepwise, while the other components were kept constant. The purpose of these experiments is, in the first place, to elucidate how the salts of a suitably constituted culture solution shall be composed. The intention in this connection is consequently not to investigate the importance of the individual ions for the growth of *Ankistrodesmus*, even if certain conclusions may be obtained in this respect too. In order to unravel such questions in a valid way a considerably larger scheme of experiments is required than the one carried out here. For investigations of that kind with *Scenedesmus quadricauda* I shall give an account in the following sections.

It should further be said that we here want to obtain an idea of the concentrations giving optimal growth; they are not necessarily identical with the concentrations giving maximal yield at a certain time, e. g. after the cessation of the growth.

For each series of experiments a culture solution was prepared which, with the exception of the salt which should be added, was composed according to the formula given above. The solution was autoclaved and then inoculated with *Ankistrodesmus falcatus* (from a greenish pure culture in medium V) by an amount which was adjusted so that the cell concentration of the solution was about 10 cells per mm³. From this 10 ml were pipetted under sterile conditions to the requisite number of sterile culture tubes, to which the remaining salt was added in graded amounts (< 1 ml) of appropriate stock solutions. In order to avoid different dilutions, as much distilled water was added to each tube as was required to ensure that the added volumes should be the same in all the tubes of the series. After determining the extinction value of each tube at the start of the experiment (which was subtracted from the values subsequently obtained, cf. p. 11) the cultures were placed in a photothermostat at 25° C with illumination for 10 hours daily. The tubes were mutually interchanged every day in order to obtain on the average the same amount of light.

The series of experiments were made on three different occasions. The

results for K_2HPO_4 , NH_4Cl , $Ca(NO_3)_2$, $MgSO_4$, Na_2SiO_3 , $CaCl_2$, and $NaHCO_3$ were obtained in a joint experiment (No. 218), while the series given for ferric citrate, citric acid, and trace elements (given as "A-Z-solution", cf. p. 56) were carried out at different times (expts 175 and 202). For all these series, the conditions of the cultures were, however, as far as possible identical.

We now proceed to a discussion of the experimental results given in Table 8.

Dipotassium phosphate.

The dependence of the rate of growth and final yield on the concentration of K_2HPO_4 is shown in fig. 8 on the basis of the values in Table 8 A (for culture 4 the extinction value Z after the eighth day is no longer a reliable measure of the substance production because the algae were then partly adhering to the walls, especially the bottom of the tube). 0.56 mg K_2HPO_4 during the first six days gives as equally rapid growth as the higher concentrations but after that is soon used up. In an analogous way both of the two following cultures (with 1.12, and 2.80 mg/l) deviate after eight days from the curve for the highest phosphate concentration which reaches its maximum after 15 days from the beginning of the experiment.

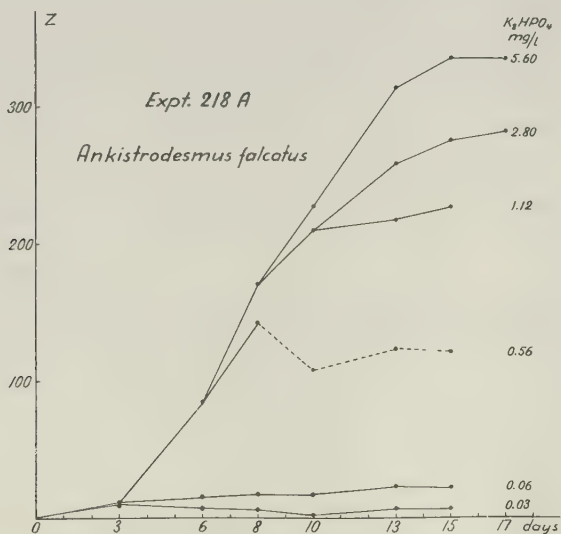


Fig. 8.

Growth (extinction values, Z) of *Ankistrodesmus falcatus* for different concentrations of dipotassium phosphate.

Table 8. Growth (Z-values) of *Ankistrodesmus falcatus* for different additions of the salts of culture solution V (only one salt is varied in each series).° = culture $\pm$ yellow.* = algae $\pm$ adhering to the walls.† = precipitation of $\text{Fe}(\text{OH})_3$ (in series G).

A. Dipotassium phosphate.

No.	$\mu\text{g P/l}$	$\text{mg K}_2\text{HPO}_4/\text{l}$	3 days	6 days	8 days	10 days	13 days	15 days	17 days
1	0	0	5	4	5	2	5	7	—
2	5	0.028	10	7	6	2	7	7	—
3	10	0.056	12	15	18	17	23	22	—
4	100	0.560	12	83	142	108°	123°	121°*	—
5	200	1.120	10	82	169	209	217°	226°	—
6	500	2.800	13	81	169	208	258	275	282
7	1,000	5.600	11	71	168	227	313	335	335

B. Ammonium chloride.

No.	mg N/l	$\text{mg NH}_4\text{Cl/l}$	3 days	6 days	8 days	10 days	13 days	15 days	17 days
1	0.0	0.0	8	9	13	7	11	8	—
2	0.01	0.04	9	9	9	8	15	11	—
3	0.05	0.19	13	20	20	16	23	19	—
4	0.10	0.38	12	16	12	8	14	10	—
5	0.50	1.91	14	68	56	°52*	°57*	°46*	—
6	1.00	3.82	14	111	85*	°59*	°62*	°49*	—
7	5.00	19.10	14	107	186	241	311	322	352
8	10.00	38.20	13	92	163	205	271	286	316

C. Calcium nitrate.

No.	mg N/l	$\text{mg Ca}(\text{NO}_3)_2/\text{l}$	3 days	6 days	8 days	10 days	13 days	15 days	17 days	25 days
1	0.0	0.0	9	12	12	7	12	9	—	—
2	0.01	0.06	9	13	12	7	12	8	—	—
3	0.05	0.29	12	22	22	12	17	19	—	—
4	0.10	0.59	10	22	18	16	25	22	—	—
5	0.50	2.94	11	73	66	°52*	°52*	°49*	—	—
6	1.00	5.88	10	72	147*	°107*	°91*	°83*	—	—
7	5.00	29.40	13	104	232	334	379	325*	317*	—
8	10.00	58.80	14	113	236	364	495	609	720	805*

Table 8 (cont.)

D. Magnesium sulphate.

No.	mg MgSO ₄ /l	3 days	6 days	8 days	10 days	13 days	15 days	17 days
1	0.0	5	27	56	°39*	°38*	°42*	—
2	0.1	4	31	95	°91*	°52*	°64*	—
3	0.5	8	41	125	178	218	192	204
4	1.0	8	37	110	177	259	287	305
5	5.0	6	37	115	178	252	263	258
6	10.0	7	39	107	165	235	255	242
7	25.0	9	50	134	188	254	258	236
8	50.0	9	59	135	188	256	263	247*

E. Sodium silicate.

No.	mg Na ₂ SiO ₃ /l	3 days	6 days	8 days	10 days	13 days	15 days	17 days
1	0.0	5	9	41	72	114	129	141
2	0.1	4	21	58	85	118	130	141
3	1.0	6	24	62	94	135	150	167
4	5.0	4	19	54	96	147	168	189
5	10.0	5	35	88	139	201	225	255
6	20.0	9	52	122	177	256	292	316
7	50.0	8	52	140	232	317	283*	282*

F. Calcium chloride.

No.	mg Ca/l	mg CaCl ₂ /l	3 days	6 days	8 days	10 days	13 days	15 days	17 days
1	0.0	0.0	4	12	35	64	117	149	174
2	0.01	0.03	3	12	39	68	117	143	166
3	0.05	0.14	3	13	38	72	127	155	179
4	0.10	0.28	4	17	47	85	138	164	187
5	0.50	1.38	5	22	61	103	155	175	198
6	1.00	2.77	3	8	27	53	118	148	179
7	5.00	13.85	5	24	63	103	156	171	195
8	10.00	27.70	8	41	91	124	165	183	201

Table 8 (cont.)

G. Ferric citrate (with equal amounts of citric acid).

No.	mg Fe/l	mg ferric citrate per litre	5 days	7 days	9 days	12 days	14 days	16 days	19 ¹ days	21 days	28 days
1	0.0	0.0	4	1	1	-1	-2	-5			
2	0.1	0.57	57	143	217	299	263	264*			
3	0.2	1.14	38	128	203	286	274	281*			
4	0.4	2.28	51	148	219	307	306	315*	332*	337*	399*
5	0.6	3.43	69	156	224	314	305	333*	321*	307*	334
6	0.8	4.57	63	148	211	294	308	345*	344*	340*	388*
7	1.0	5.71	50	139	206	290	308	347*	341*	353*	445*
8	2.0	11.42	49	136	196	273	291	341*	373*	407*	522*
9	4.0	22.84	53	110	153	216	220	262*			
10	6.0	34.26	12†	42†	91†	156†	168†	205†*			
11	8.0	45.68	14†	34†	68†	128†	142†	174†*			
12	10.0	57.10	16†	18†	33†	86†	106†	140†*			

¹ On the nineteenth day of the experiment the following additions were made (in quantities corresponding to the original concentration of culture solution V):

No. 4: K_2HPO_4 ,

No. 5: NH_4Cl ,

No. 6: ferric citrate-citric acid,

No. 7: 1.0 ml "A-Z-solution" diluted 100 times,

No. 8: no additions.

H. Ferric citrate (with equal amounts of citric acid).

No.	μg Fe/l	μg ferric citrate per litre	4 days	6 days	8 days	10 days	13 days
1	0	0	7	9	11	8	10
2	1	5.7	9	12	13	12	12
3	2	11.4	8	10	12	11	13
4	4	22.8	13	22	93	177	282
5	6	34.3	13	40	125	198	286
6	20	114	25	74	170	230	304
7	40	228	30	100	191	258	316
8	60	343	38	115	200	258	312
9	80	457	37	115	196	257	319
10	100	571	38	110	188	246	307

Table 8 (cont.)

I. Citric acid.

No.	mg citric acid per litre	initial pH	4 days	6 days	8 days	10 days	13 days
1	0	5.9	30	82	170	224	302
2	1	6.1	20	47	119	177	246
3	2	—	23	52	121	165	201
4	6	—	31	75	149	189	253
5	10	6.1	30	74	141	176	232
6	20	6.0	19	35	70	93	119
7	60	5.4	5	5	8	7	8
8	100	4.6	7	8	11	10	11

K. Sodium bicarbonate.

No.	mg NaHCO ₃ /l	initial pH	3 days	6 days	8 days	10 days	13 days
1	0	7.4	9	76	160	212	221
2	50	7.5	14	114	248	277	287
3	100	7.6	13	160	336*	287*	255*
4	150	7.8	14	174	338	254*	248*
5	200	7.9	13	167	329*	251*	208*
6	250	8.0	10	179	331*	254*	315*
7	350	8.1	9	167	318	250*	272*
8	500	8.3	9	168	303	302*	307*

L. Trace elements ("A-Z-solution").

No.	ml "A-Z" per litre	4 days	6 days	8 days	10 days	13 days
1	0.0	22	50	131	189	233
2	0.05	24	65	144	197	262
3	0.1	23	60	143	190	251
4	0.3	19	53	126	177	242
5	0.5	24	53	126	178	242
6	0.6	23	54	129	180	249
7	0.7	21	54	130	185	250
8	0.8	21	52	134	188	253
9	1.0	21	47	119	176	247

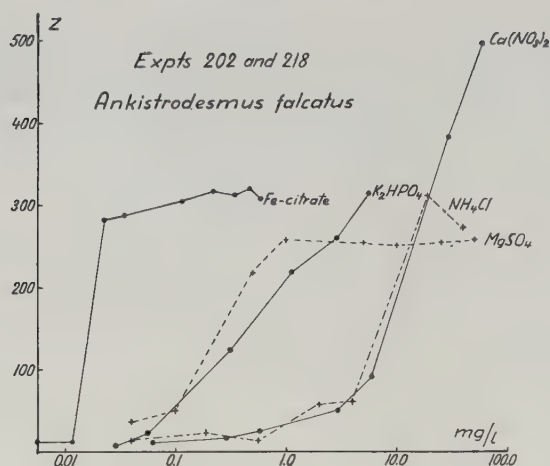


Fig. 9.

Yield curves (after 13 days) of *Ankistrodesmus falcatus* for varying amounts of nutrient salts (plotted on a logarithmic scale). Refer to Table 8.

The yield curve for the thirteenth day (fig. 9) shows by its course that yet higher productions would certainly have been obtainable with a higher phosphate addition than 5.6 mg K_2HPO_4/l .

The curves in fig. 8 further show that the final yields are not proportional to the amounts of phosphate; e. g. for tubes 5, 6, and 7 the latter have the ratios 2 : 5 : 10, but the ratios of the extinction values (Z , Table 8 A) for these tubes on the fifteenth day are about 2 : 2.4 : 3. Especially for higher productions, potassium phosphate is obviously not the only limiting factor in culture solution V. That the rate of growth is also affected by other factors than the amounts of added phosphate and that this affect takes place even during the first days of the experiment follows from a comparison with the series in which $Ca(NO_3)_2$ replaced NH_4Cl as nitrogen source (Table 8 C). There both growth and final yield are considerably higher than for the highest phosphate concentration (Table 8 A), despite the content of K_2HPO_4 being somewhat lower (5.0 mg/l according to the formula for culture solution V). This shows that the results obtained strictly hold only for the conditions under which they were taken. This fact, which will be discussed in the later sections (cf. p. 116), complicates, of course, the application of experiences gained for special experimental conditions and calls for great care in generalizations.

Summarizing: 1.12 mg K_2HPO_4/l (= 0.2 mg P/l and 0.5 mg K/l) is the lowest concentration examined here which, with the remaining composition according to medium V, gives maximal rate of growth of *Ankistrodes-*

mus falcatus during about eight days. By increasing the phosphate concentration, higher yields can be attained, but in culture solution V other factors then become more and more limiting so that the phosphate cannot be completely utilized.

Ammonium chloride and calcium nitrate.

The assimilation of ammonium and nitrate by algae (especially by Chlorophyceae such as *Chlorella* and *Scenedesmus*) has been the object of many investigations. An account of the partly contradictory results (see, e. g., ALGÉUS 1946 and the literature quoted there) falls outside the scope of the present position of the problem. It is sufficient to state here that the algae in general seem to be able to utilize both ammonium salts and nitrates as inorganic sources of nitrogen, although one often considers to have found a certain predilection for one kind or another (concerning plankton algae see CHU 1943).

The difference between ammonium and nitrate nutrition appears particularly in highly productive algal cultures by secondary effects in connection with the hydrogen ion concentration of the solutions. Thus on the consumption of the nitrogen the ammonium salts give a decrease in the pH while the nitrates give an increase. Besides, the former, for alkaline reaction, give free ammonium hydroxide, which is harmful for the continued growth of the algae, destroying their chlorophyll (ÖSTERLIND 1947).

In culture solution V, NH_4Cl is used as nitrogen source. On the ground of the suspicion that the unsatisfactory results with this medium would depend on this to an essential degree, parallel series were started with NH_4Cl and $\text{Ca}(\text{NO}_3)_2$. The salts were added with equal nitrogen concentrations (Table 8 B and 8 C).

In both series concentrations up to 1 mg N/l were apparently insufficient, while 5 mg N/l gave better results. At this concentration about the same final yield was obtained for ammonium and nitrate, but the rate of growth was better in the nitrate series. For the highest nitrogen content, 10 mg/l, both growth and final yield in the NH_4 series became worse than with 5 mg/l. Both the results of ÖSTERLIND (unpublished) and my own later experiences have shown that the intensive CO_2 assimilation in highly productive cultures of *Ankistrodesmus* and *Scenedesmus* can lead to an increase of pH to 11 or yet higher. With the use of an ammonium salt as nitrogen source the growth is however stopped on the ground of the formation of NH_4OH .

On the other hand with calcium nitrate a doubling of the nitrogen content from 5 to 10 mg N/l caused a doubling of the final yield (Table 8 C),

and judging from the yield curve in fig. 9 yet higher yields could have been obtained by further increase of the nitrate concentration. The proportionality between the nitrogen additions and the corresponding yields shows that other factors do not here have a limiting effect. The limiting of the yield in the phosphate experiment (at 5.25 mg $\text{N}(\text{NH}_4)/\text{l}$) stated above apparently depended on the nitrogen factor.

Although the final yield was doubled, no difference between 5 and 10 mg $\text{N}(\text{NO}_3)/\text{l}$ exists with respect to the rate of growth during the first eight days. The former concentration (29.4 mg $\text{Ca}(\text{NO}_3)_2/\text{l}$) is consequently the lowest of those tested here which can give, during a period of one week, optimal growth with *Ankistrodesmus falcatus* in culture solution "V without NH_4Cl ."

Magnesium sulphate.

For 0.1 mg MgSO_4/l (Table 8 D) only insignificant growth is obtained and after only eight days the algae adhere to the walls. On the other hand, with 0.5 mg/l, optimal rate of growth is obtained during the first ten days, and with 1—50 mg/l the final yield is about the same in all the cultures (fig. 9).

Sodium silicate.

The addition of sodium silicate (Table 8 E) is clearly not necessary for growth to take place, but a positive effect is observed for concentrations up to 20 mg/l. This effect probably depends on the weakly alkaline reaction of sodium silicate which is favourable for *Ankistrodesmus* (as for *Scenedesmus quadricauda*, cf. ÖSTERLIND 1947).

Calcium chloride.

In order to decide whether $\text{Ca}(\text{NO}_3)_2$ could have any effect by its calcium content and if CaCO_3 in culture solution V fulfils any function as calcium source, a series of experiments was carried out with CaCl_2 (Table 8 F). Additions of different amounts of CaCl_2 had however no significant effect on either the rate of growth or the yield. Other than the small amounts of Ca and Cl which had possibly entered as impurities in the other salts, no addition of these ions was therefore necessary for *Ankistrodesmus falcatus*.

Ferric citrate.

In a first experiment (Table 8 G), ferric citrate was added (with equal amounts of citric acid) for Fe-concentrations from 0.1—10.0 mg/l. While

growth almost completely failed to appear for 0.0 mg Fe/l, even the lowest iron content, 0.1 mg Fe/l, proved to give maximal growth and almost maximal yield. For this cause a new series was started with additions from 1 to 100 μ g Fe/l (Table 8 H). 2 μ g Fe/l had no significant effect, but only 4 μ g Fe/l gave a particularly apparent growth; after thirteen days almost maximum yield was obtained (fig. 9). The highest rate of growth was reached for 40 μ g Fe/l or more. Table 8 G shows that the optimum interval extended up to a 50 times higher concentration, 2 mg Fe/l, above which an inhibition was noticeable, especially for concentrations $\geq$ 6 mg Fe/l where precipitation of $\text{Fe}(\text{OH})_3$ took place.

When the growth ceased in the first ferric citrate experiment, some of the salts present in culture solution V were added to some of the cultures with yellow algae more or less adhering to the walls, in amounts corresponding to the respective original concentrations (see the note in Table 8 G). A positive effect was only obtained for NH_4Cl , which after two days restored the green colour to the culture. This confirms our result obtained above that the concentration of nitrogen in culture solution V is not satisfactory and for higher production constitutes a limiting factor.

Citric acid.

Citric acid was added in amounts equal to the ferric citrate. The series given in Table 8 I shows that no definite effect of the addition of citric acid can be traced, before its concentration increases to > 10 mg/l. It is probable that the inhibition which then sets in depends on the reduction of the pH by the citric acid. Up to about 2 mg Fe/l (= 11.4 mg ferric citrate/l and the same concentration of citric acid) one can thus add the iron as ferric citrate—citric acid, without needing to risk any appreciable effect of the citric acid on growth.

Bicarbonate.

In most natural waters the HCO_3 ions play a quantitatively dominating role and the bicarbonate can as a rule be made use of as carbon source for the assimilation of carbonic acid (see e.g., ÖSTERLIND 1947, 1948, STEEMANN NIELSEN 1947). Culture solution V contains, however, only 12 mg HCO_3 /l. In order to investigate the effect of larger concentrations of bicarbonate on the growth of *Ankistrodesmus*, two series were carried out with additions of NaHCO_3 and KHCO_3 respectively. They gave concordant results, and therefore it is sufficient to give only one of them (Table 8 K).

A comparison with the highest Z values in the other series (Tables 8 A—I) shows that the additions of bicarbonate considerably increased the

rate of growth during the beginning of the experiment. But after 10 days the algae became yellow and adhered to all the tubes which contained more than 50 mg NaHCO_3/l . This degeneration is, however, an effect of the ammonium nitrogen; it fails to appear if one uses nitrate instead (cf. p. 113, expt. 980 B). With nitrate as nitrogen source one can attain very rapid growth up to particularly high production by the addition of bicarbonate (ÖSTERLIND 1947). One can obtain a similar result by directly increasing the free CO_2 content of the culture solution, but in such a case one must either close the culture vessel hermetically or also arrange for continued bubbling with carbon dioxide.

Trace elements.

By addition of 100 times diluted "A-Z-solution" according to HOAGLAND (SCHROPP & SCHARRE 1933), 9 culture tubes containing 10 ml of culture solution V received different concentrations of a mixture of the most important trace elements (Al, I, Br, Ti, Sn, Li, Mn, B, Zn, Cu, Ni, Co). These additions had no effect whatever on the growth of *Ankistrodesmus* (Table 8 L). Neither did I find any effect if the manganese addition to culture solution V was left out, which was done in a parallel experiment (not recorded in the table).

The preparation of the final nutrient solution.

On the basis of the experimental results given above we could first state that a culture solution suited for *Ankistrodesmus falcatus* should contain:

- a) at least 1.12 mg $\text{K}_2\text{HPO}_4/\text{l}$ (= 0.5 mg K/l and 0.2 mg P/l)
- b) at least 29.4 mg $\text{Ca}(\text{NO}_3)_2/\text{l}$ (= 7.2 mg Ca/l and 5.0 mg N/l)
- c) at least 0.5 mg MgSO_4/l (= 0.1 mg Mg/l and 0.4 mg SO_4/l)
- d) at least 0.23 mg ferric citrate/l (= 0.04 mg Fe/l)
- e) preferably 20 mg $\text{Na}_2\text{SiO}_3/\text{l}$ (= 7.5 mg Na/l and 4.6 mg Si/l).

Strictly speaking these values — as already pointed out — only hold for the conditions under which they were obtained (culture solution V). It seems, however, justified to proceed from the given orders of magnitude for the composition of a similar but better culture solution.

The primary purpose of this, according to the line of thought developed in the introduction, is to offer as many different plankton algae as possible a suitable environment. The concentration of the various constituents as well as the total electrolyte content of the solution and the pH should there-

fore to the greatest extent possible agree with the common conditions in nature. For Ca, Mg, Na, K, SO_4 , Fe, and Si, the minimum limits given above lie in or partly somewhat under the respective ranges of concentration for ordinary lake waters. This is not the case with phosphate and nitrate where the minimum culture values lie a long way above the concentrations occurring in nature. Such large amounts of phosphate and nitrate as would be required to give maximal yield with, e. g., *Ankistrodesmus falcatus*, would not be advisable in the culture solution planned here. Neither is the addition of bicarbonate suitable, because very large amounts would be required in order to prevent, by means of buffering, the pH increase following upon high production. In the cases where the largest possible yield is wished in the shortest possible time for an alga which already grows in a synthetic culture solution, phosphate, nitrate, and possibly bicarbonate may be supplied additionally according to the need.

Ankistrodesmus falcatus did not require extra addition of manganese, but it should nevertheless be suitable to include a manganese salt in the culture solution in order to provide for algae with a possibly higher manganese requirement.

As a result of the experiments cited and the recently developed points of view, the following culture solution with the designation VIII was composed (August 1944):

Culture solution VIII.

	mg/l		mg/l	mg/ml in stock solu- tion	ml of stock solution per litre medium
Ca	14.7	$\text{Ca}(\text{NO}_3)_2$	60.0	10	6.0
Mg	1.0	MgSO_4	5.0	1	5.0
Na	7.5	Na_2SiO_3	20.0	1	20.0
K	2.2	K_2HPO_4	5.0	1	5.0 ¹
SO_4	4.0	MgSO_4	5.0		
N	10.2	$\text{Ca}(\text{NO}_3)_2$	60.0		
P	0.89	K_2HPO_4	5.0		
Fe	0.18	Ferric citrate — citric acid	1.0+1.0	0.1+0.1	10.0 ²
Mn	0.01	MnSO_4	0.03	0.1	0.3
Si	4.6	Na_2SiO_3	20.0		

¹ The phosphate stock solution is autoclaved separately and added to the culture solution after its autoclaving; otherwise precipitation (calcium phosphate?) can accidentally appear after autoclaving.

² The iron stock solution is likewise autoclaved separately and added to the culture solution after its autoclaving; otherwise the ferric citrate is hydrolysed, the iron being precipitated as $\text{Fe}(\text{OH})_3$.

The pH of the culture solution varies somewhat from time to time owing to the weak buffering; after autoclaving the pH usually lies at 7.0—7.5. The specific conductivity (20° C) is $125\text{--}130 \times 10^{-6}$. Both pH and conductivity thus correspond to conditions often occurring in nature. It can be mentioned that the lake Erken, from which both the strain of *Ankistrodesmus* used here and many other of the algae mentioned below derive, usually has a pH between 7.0 and 8.2 and a conductivity between 250 and 260×10^{-6} for the surface water.

Positive results.

In culture solution VIII, I have cultivated with good results a number of different plankton algae during the past years. The following cultures are even now kept continuously by reinoculation about once every six weeks. The initial cell or colony was isolated under sterile conditions with a micro-manipulator, mainly during the years 1944 and 1945.

	Number of strains
<i>Ankistrodesmus falcatus</i> (CORDA) RALFS.....	2
» <i>nannoselene</i> SKUJA n. sp.....	1
<i>Chlorella ellipsoidea</i> GERNECK.....	1
» <i>pyrenoidosa</i> CHICK.....	4
» <i>vulgaris</i> BEYERINCK.....	1
<i>Coccomyxa coccoides</i> RODHE et SKUJA n. sp.....	2
<i>Coelastrum microporum</i> NAEGELI.....	3
<i>Dictyosphaerium pulchellum</i> WOOD.....	1
<i>Pediastrum biradiatum</i> MEYEN.....	2
» <i>Boryanum</i> (TURPIN) MENEGH. (typicum).....	1
» » fa. <i>clathrata</i> n. fa. biologica ad interim.....	1
» » var. <i>rugulosum</i> G. S. WEST.....	3
» <i>duplex</i> MEYEN (typicum).....	1
» » var. <i>reticulatum</i> LAGERH.....	1
<i>Scenedesmus acutus</i> (MEYEN) CHOD.....	4
» <i>armatus</i> CHOD.....	2
» <i>costato-granulatus</i> SKUJA n. sp.....	1
» <i>ecornis</i> (RALFS) CHOD.....	2
» fa. ad <i>intermedius</i> CHOD.....	1
» <i>Iovis</i> CHOD.....	1
» <i>Naegelii</i> BRÉB. sensu SMITH.....	2
» <i>quadricauda</i> TURPIN em. CHOD.....	3
<i>Chlamydomonas botrys</i> RODHE et SKUJA n. sp.....	1
» <i>gloeopara</i> RODHE et SKUJA n. sp.....	1

	Number of strains
<i>Eudorina elegans</i> E.....	2
<i>Gonium pectorale</i> MÜLLER.....	1
<i>Staurastrum gracile</i> RALFS fa. ad var. <i>nanum</i> WILLE.....	2
» » » fa. ad var. <i>coronulatum</i> BOLDT.....	1
<i>Xanthidium antilopaeum</i> (BRÉB.) KG. var. <i>dimazum</i> NORDST. fa. CEDERGREN	1
<i>Tribonema ambiguum</i> SKUJA n. sp.....	1
<i>Asterionella formosa</i> HASSALL.....	4
<i>Tabellaria flocculosa</i> (ROTH) KG.....	1

Of pure cultures which during a longer time showed growth in culture solution VIII but then for different reasons (bacterial or fungal infection, delayed reinoculation, accidents, etc.) died away, the following could be named.

Pandorina morum BORY

Closterium aciculare T. WEST

Staurastrum pseudopelagicum W. et G. S. WEST var. *tumidum* G. M. SMITH

Diatoma elongatum AG.

Fragilaria capucina DESM.

» » var. *mesolepta* (RBH.) GRUN.

» *crotonensis* KITTON

Melosira islandica O. MÜLL. subsp. *helvetica* O. MÜLL.

» *varians* AG.

Nitzschia parvula LEWIS

Synedra ulna (NITZSCH) E. var. *danica* (KG.) GRUN.

The determination of the algae has been verified and also partly carried out by H. SKUJA, who describes the new species stated above in his monograph on the taxonomy of plankton algae (SKUJA 1948).

These positive results show that solution VIII is applicable as culture medium for species belonging to Chlorococcales, Volvocales, Conjugatae, Heterocontae, and Diatomaceae. The ionic concentrations of this medium thus seem to be suitable — or in any case acceptable — for many of the most common plankton algae.

A comparison of the composition of medium VIII with the results obtained by CHU (1942, 1943) is interesting. He found that the best concentrations of phosphate-phosphorus and nitrate-nitrogen in culturing some plankton algae were the following:

	mg P/l	mg N/l
<i>Pediastrum Boryanum</i>	0.09— 1.9	1.3—13
<i>Staurastrum paradoxum</i>	0.1 — 1.8	2 —10
<i>Botryococcus Braunii</i>	0.09—18	1.4—14
<i>Nitzschia palea</i>	0.2 — 1.8	5 —10.5
<i>Fragilaria crotonensis</i>	0.2 — 1.8	1.7—10.5
<i>Asterionella gracillima (formosa?)</i>	0.18— 9	1.7—17
<i>Tabellaria flocculosa</i>	0.2 — 1.8	

compare with,

culture solution VIII.....	0.89	10.2.
----------------------------	------	-------

Concerning the remaining constituents, the account of CHU is not equally comprehensive, but even here the respective ranges of concentration for the best growth of the investigated algae seem to a large degree to be approximately equivalent to the values which hold for culture solution VIII. It is perhaps therefore no accidental circumstance that this culture solution and that of CHU's solutions which he himself found to be most suitable for different objects, namely his Number 10, agree fairly well in their general composition.

The medium Chu 10 diverges, however, at an essential point from my culture solution VIII — in the source of iron. Thus CHU uses, as in his other solutions, FeCl_3 as iron source. But the iron in this form, as I have already shown (p. 33), is particularly unstable in solution, especially after autoclaving. For many parallel cultivations with Chu 10 and Rodhe VIII the latter proved to be decidedly superior, until I exchanged the FeCl_3 in Chu 10 for the corresponding amount of ferric citrate — citric acid.

Negative results and their causes.

The account of culture solution VIII and its applicability would be incomplete if I did not give at least an intimation of the numerous experiments with this culture solution which have given negative results. It is probably significant that I did not fail with a single one of all those Chlorococcales which I have purposely tried to cultivate in medium VIII, while on the other hand I have not yet — despite persistent efforts and systematic variation of the experimental conditions — obtained a single plankton alga belonging to Cyanophyceae, Chrysophyceae, Cryptophyceae, or Dinophyceae in pure culture on a completely synthetic basis.

The common planktonic Cyanophyceae belonging to the genera *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, and *Microcystis* in particular have

been objects for many experiments of different kinds, but none of the ways tried has up to now led to the aim — continuous growth in completely synthetic environment. Of Chrysophyceae I have tried to cultivate *Synura uvella*, and of Cryptophyceae some species of *Cryptomonas*. Among the diatoms, *Melosira granulata* and *Stephanodiscus astraea* presented difficulties to me. For these algae, culture solution VIII is obviously directly unsuitable, and the causes for this, which without doubt can alter from case to case, may be of two different categories:

- a) the solution contains *too much* of one or several constituents (for example, too much phosphate for certain Chrysophyceae, cf. below p. 90);
- b) the solution lacks or contains *too little* of one or several necessary substances. This applies in the first place to organic substances, which (with the exception of ferric citrate and citric acid) are completely missing from culture medium VIII.

By the addition of extract of soil or of lake sediment (the extracts prepared by boiling) to medium VIII, I have in many cases, e. g., with *Synura uvella*, *Cryptomonas ovata*, *Melosira granulata*, and *Stephanodiscus astraea*, obtained growth during at least a few weeks or months. In parallel experiments with additions of corresponding amounts of redissolved ash of the extracts no positive results were obtained. Since the effect of the extracts in culture solution VIII can scarcely have any connection with the iron requirement, it must depend on organic thermostable substances which are required for the growth of the plankton algae mentioned. In that case these should be mixotrophic, but one can distinguish between obligative and facultative mixotrophes, and certain observations suggest that the species mentioned are rather to be considered as facultative mixotrophic algae.

This is, however, scarcely the case with the planktonic Cyanophyceae with which, especially, I have carried out many negative experiments. Despite different additions of soil or sediment decoctions it proved to be impossible in culture solution VIII or any other synthetic medium to obtain any growth worth mentioning of, e. g., *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, *Coelosphaerium Naegelianum*, or *Microcystis aeruginosa*. Therefore I proceeded to experiments using fresh lake water (from Erken) as medium. Different external conditions (e. g., different temperature and light conditions, continuous shaking or stirring etc.) and different additions (inorganic salts, organic phosphorus and nitrogen compounds, extracts of soil or sediment) were tried in various combinations but the result was nevertheless finally always the same: cessation of growth.

In these experiments I found that *Gloeotrichia echinulata* was a parti-

cularly suitable experimental object among the planktonic Cyanophyceae. Its globular colonies in healthy state have a beautiful radiating "corona." But, with inability to thrive, they alter their macroscopic appearance on the basis of the rapid breakdown of the trichomes: the "corona" gradually disappears and the *Gloeotrichia* globule finally obtains a stubbed outline. These changes can easily be observed with a hand lens or simply with the naked eye.

With *Gloeotrichia* I made new culture experiments at the Erken laboratory during the summer of 1946. The earlier negative results were repeated, but I could state that Erken water seemed to contain a factor the presence of which was necessary for the *Gloeotrichia* globule not to degenerate. This factor seemed to be very unstable or insufficient, because not once in fresh water samples could I get a *Gloeotrichia* colony to form more than a few daughter globules, before the algae degenerated in the usual manner. This, despite the fact that *Gloeotrichia* was developing at the same time a high production in Erken.

But at the beginning of August, for lack of a better idea, I carried out the following simple experiment. To each of three culture tubes containing 10 ml of fresh Erken water was added 1 g of fresh garden soil and then a *Gloeotrichia* colony. After one week all the algae were not only alive but in all the tubes the originally single globule had multiplied to about twenty colonies. After another week each tube contained about fifty *Gloeotrichia* globules in a very beautiful state.

On the ground of this first success, three new series of experiments were started with different amounts of Erken water (10, 20, and 30 ml) to 1 g of garden soil. The day before the inoculation with one *Gloeotrichia* globule per tube, the tubes were treated in each series in the following way:

series I: soil + water, no further proceedings,

series II: soil + water, tubes placed in boiling water for 1 hour,

series III: before the addition of water the soil was heated for 1 hour at about 175° C.

Only one day after inoculation, all the *Gloeotrichia* globules in series II and III were degenerating, while those in series I and in the control samples (with Erken water only) were in a good state. The following day the colonies in the control samples showed (as usual!) obvious signs of degeneration and after a further day they were disintegrating. On the other hand, in series I the increase was well under way in all the tubes but best in that which had only 10 ml of Erken water. A week after the start the increase had gone yet further and one could then easily distinguish the order of rank: 10 ml > 20 ml > 30 ml. At the same time a certain degeneration

of the *Gloeotrichia* globules, with the formation of akinetes, began to assert itself in all the tubes, and a few days later the globules lay at the bottom in a disintegrated condition. Since at this time (end of August) *Gloeotrichia* even in Erken decreased strongly in numbers and began to form akinetes, the experiment could not be continued.

It seems, however, to follow even from this preliminary experiment that *Gloeotrichia* requires for its growth access to one or several organic substances which are not thermostable. Consequently it must here be a question of other substances than those which are obtained in decoctions of soil or lake sediment. On the contrary, when soil is heated such substances seem to be set free or to be formed as are harmful for *Gloeotrichia* which in this respect is distinguished from many other algae. But I suspect that the other planktonic Cyanophyceae have approximately the same characteristic claims as *Gloeotrichia* and that this is the reason why up to now I have never succeeded in cultivating any of these algae in continuous culture.

Shortly after these experiments had been made I learnt of PRINGSHEIM's fine results (1946 a, 1946 b) with the cultivation of many algae so far difficult to cultivate. He introduced a new technique, "the biphasic or soil-water culture." By the positive results with *Gloeotrichia echinulata* I can confirm the value of this method. Starting from soil-water cultures, it should be possible to investigate more closely the requirements of the mixotrophic algae. Not the least concerning plankton algae many important problems await solution here.

With culture solutions of the inorganic type, as my culture solution VIII represents, only autotrophic plankton algae can be cultivated. However, even their physiology and ecology are still very incompletely known. The best way to fill up the gaps in our knowledge seems to me to be a consistent combination of experimental investigations and ecological observations. I hope to show this to some degree in the following sections.

V. Phosphate as a Factor for Growth.

Introduction.

Phosphorus belongs to the elements necessary for life. The phosphorus content of lake waters is generally very low, and according to the principles of regional limnology (NAUMANN 1932) the phosphorus supply is one of the deciding factors for the phytoplankton production. For lakes in Connecticut, U. S. A., DEEVEY (1940) obtained a very close correlation, $r = + 0.919$, between the total phosphorus content and the chlorophyll content (which was used as a measure of phytoplankton). This connection, which is elucidable (but partly contradictable also) by numerous other quotations from the limnological literature, is, however, very summary in character, because neither total phosphorus nor phytoplankton is a unitary conception. A thorough study of the importance of the assimilable phosphorus for the growth of the individual plankton algae is therefore necessary.

In this respect little information has hitherto been obtainable. Only CHU (1942, 1943) has experimentally studied the effect of different phosphate concentrations on the growth of a number of fresh-water plankton algae. He found (1943 p. 147) that "the lower limit of the optimum range of phosphorus concentration varies from about 0.018 (*Nitzschia palea*, *Ta-bellaria flocculosa*) to about 0.09 (*Pediastrum*, *Staurastrum paradoxum*, *Botryococcus*) p. p. m." The phosphate requirement of the algae investigated seems to lie considerably above the concentrations which usually exist in the epilimnion of lakes. CHU's results thus give support to the conception that the phosphate content is a minimum factor for the production of phytoplankton.

My own investigations of the phosphate dependence of growth for some plankton algae have not in all respects given definite results, but they seem to show clearly that the role of the phosphate as a limiting factor for the growth of the different plankton algae in the lakes is considerably more diverse and more complicated than had been hitherto conceived.

Concerning *Scenedesmus quadricauda*.

Scenedesmus quadricauda TURPIN em. CHOD. can easily be cultivated in synthetic culture solutions. Since 1944 I have kept some bacteria-free pure cultures (clones) of this plankton alga, originating from the lake Erken, permanently growing in culture solution VIII. In this culture medium, however, the phosphate concentration is 0.89 mg $P(PO_4)/l$, i. e., about 40 times higher than the total phosphorus content in the original environment of the alga. To establish approximate agreement in this matter, I made up a modified culture solution, called VIII (P = 20), containing only 20 μg P/l as K_2HPO_4 . 5.0 mg K/l were added as KCl, in order to avoid potassium deficiency (cf. p. 118).

Growth of *Scenedesmus* was also obtained in VIII (P = 20), although it was considerably slower than in medium VIII. After cultivation for three months in two successive cultures, material from VIII (P = 20) — the algae separated from the solution by centrifuging — was inoculated into 100 ml of completely phosphate-free culture solution, called VIII (P = 0), which was divided between 10 sterile culture tubes with 10 ml in each. By additions of K_2HPO_4 solutions (and dist. water to give the same total volume in all the tubes) the following concentrations were then obtained:

0, 10, 20, 30, 40, 50, 100, 200, 500, and 1,000 μg P/l.

The tubes were photometrically measured to establish the extinction value at the start and placed in a photothermostat at 25° C with 20 hours' illumination per day. The cell concentration was determined at the start to be about 38 cells/mm³. The photometric measurements and the counts were then made 2, 3, 4, 6, and 7 days after the start.

The results of this experiment (No. 982) are shown in fig. 10 (extinction values, Z) and fig. 11 (cells per mm³) in the form of time-curves. In Table 9 an extract is given of the experimental records of the cell number for the last two days of the experiment.

It appears immediately from both of the diagrams that the maximum rate of growth of *Scenedesmus quadricauda* for the prevailing conditions called for a phosphate concentration of about 1,000 μg P/l. The lowest concentrations tried here, 10—40 μg P/l, are comparable with, or a little higher than, the total phosphorus content in many natural waters. At these concentrations the growth was only a fraction of the maximum and after 6 days the cell increase completely ceased (the extinction values on the other hand continued to rise, which shows that the substance production is fairly independent of the cell divisions, cf. Fig. 4). If one compares

Table 9. Experiment 982. Number of *Scenedesmus* cells per mm³ after 6 and 7 days. Initial number 38 cells/mm³.

$\mu\text{g P/l}$	Cells per mm ³		Relative increase after 7 days
	6 days	7 days	
0	23 $\pm$ 2	20 $\pm$ 3	—
10	83 $\pm$ 10	80 $\pm$ 7	2.1
20	130 $\pm$ 14	127 $\pm$ 11	3.3
30	269 $\pm$ 31	290 $\pm$ 24	7.6
40	326 $\pm$ 25	328 $\pm$ 27	8.6
50	381 $\pm$ 44	485 $\pm$ 44	12.7
100	1,200 $\pm$ 125	1,265 $\pm$ 121	33
200		1,730 $\pm$ 73	46
500	1,990 $\pm$ 67	3,190 $\pm$ 181	84
1,000	3,420 $\pm$ 308	3,420 $\pm$ 127	90

the relative increase after, e. g., 7 days (Table 9) with the corresponding phosphate concentrations, it is found that direct proportionality holds to a large extent up to 200 $\mu\text{g P/l}$. At these concentrations the growth was thus limited in the first place by the supply of phosphate, but for higher productions one or a few other factors began to come below their optimum.

In the experiment described, the growth of *Scenedesmus quadricauda* was studied in small closed volumes of the medium. Recently, PRATT, by a series of fine investigations (see, e. g., PRATT 1942 and 1944) with pure cultures of *Chlorella vulgaris*, has been able to show that a substance called "chlorellin" is formed during growth and that this substance can have an inhibiting effect on the increases in closed volumes with high population densities. Since similar conditions probably exist in pure cultures of *S. quadricauda*, the results obtained here could possibly be influenced by an inhibiting factor in a way which jeopardizes their application in ecological connections.

Another objection against such an application is that the population density of phytoplankton under ordinary conditions in nature does not reach such high levels as are involved in pure cultures (in the preceding experiment the initial concentration was already about 38,000 cells/ml and for the highest phosphate additions the density exceeded 3 million cells/ml after 6—7 days). In a closed culture, therefore, a growth-limiting constituent, e. g., phosphate below its optimum interval, must be used up by the algae at a rate which can scarcely have its correspondence in nature. Better agreement concerning the conditions would certainly be obtained

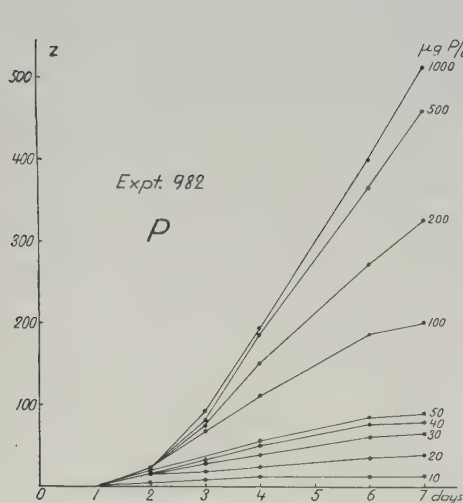


Fig. 10.

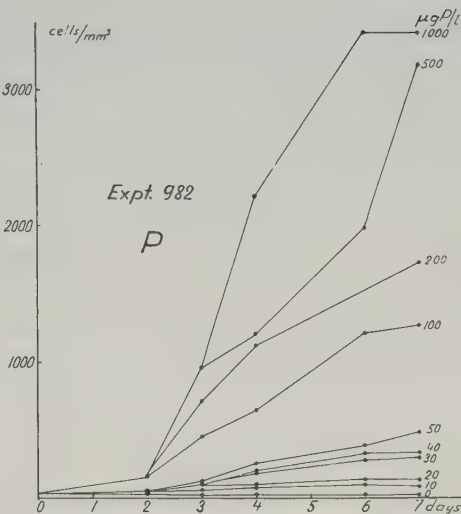


Fig. 11.

Growth (extinction values, Z, fig. 10; and cell numbers, fig. 11) of *Scenedesmus quadricauda* for different concentrations of phosphate-phosphorus.

if the composition of the culture solution could be kept constant or nearly constant during the whole of the time of the experiment. In order to provide for these points of view to the utmost possible extent, two parallel series (1020 A and 1020 B) were started in connection with the preceding experiment, the cultures partly — as before — in usual test tubes (A), and partly in "filter tubes" (B).

I originally worked out the filter tube method in order to obtain high productions of "starvation-cultures," in which the growth ceased on the ground of the lack of a single ion (cf. p. 74). The filter tubes were prepared (by the firm of R. Grave, Stockholm) by altering so-called Nutschen (Jena Filter Test Tubes) with 20 mm internal diameter. The stem of the funnel was cut off immediately below the filter plate and the upper portion was lengthened to 15 cm by fusing on a glass tube of corresponding diameter and quality. With a pore-size of 5–10 μ , the filter (type 15 a G 3) was impermeable to *Scenedesmus quadricauda* and other algae of corresponding or greater dimensions.

The arrangement of a filter culture follows from fig. 12. The algal suspension (12 ml) is within the filter tube, which by means of a cork ring is kept suspended in a wide-necked Erlenmeyer flask of 100 ml, containing 70 ml of the same culture solution. In order to avoid volume changes of the inner solution, which communicates with the outer solution via the filter, the

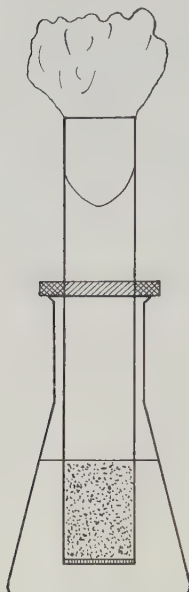


Fig. 12.

Arrangement for a filter tube culture.

position of the tube is so adjusted that the level of the liquid is at the same height inside and outside the tube.

If the concentrations of the dissolved substances for some cause are different in the inner and outer solutions, a compensation takes place through the filter plate until finally complete equality prevails. It proved, however, that a layer of, e. g., *Scenedesmus quadricauda* on the filter considerably delayed this compensation. The equalization could be accelerated by continually shaking the whole culture, but even then the diffusion did not become so rapid that it could completely compensate the consumption by the algae of, e. g., phosphate in the inner solution. In order to bring about approximately constant conditions I therefore resorted to suction filtering at short intervals (e. g., each day), whereby the inner solution was rapidly removed and replaced with a new one; at the same time the outer solution was exchanged. This method proved to be very applicable not only in maintaining the composition of the culture solution during the whole time of the experiment but also in order — when so wished — to attain an opposite result: to alter rapidly and completely the character of the medium in some respect. The reaction of the growth could be easily followed in the usual way by photometric measurements of the filter tube.

With a pore-size of 5–10 μ the filter plate, of course, offers bacteria

nearly free passage into the algal culture. Experience has, however, shown that no risk worth mentioning exists for a serious bacterial infection, if the inner and outer solutions are often changed for new and sterile ones.

We can now pass on to experiment 1020. Each of the following phosphate concentrations were added to a test tube and a filter tube: 0, 10, 20, 40, 50, and 100 $\mu\text{g P/l}$. A culture of *Scenedesmus quadricauda* in medium VIII(P = 20) was used as inoculating material, as in the experiment described above, and the initial concentration was about 25 cells/ mm^3 . The 12 cultures were placed in a photothermostat with continuous shaking at 25° C and 20 hours' illumination per day. The solutions of the filter tube cultures were changed daily (after photometric measurements, and counts) for new sterile solutions of corresponding composition. The experimental results are shown in graphical form in fig. 13 (extinction values, Z) and fig. 14 (cell number/ mm^3); in order not to complicate the survey, the middle growth curves for the test tube series have been omitted from the diagrams.

The photometric determinations and counts, as shown, give a clear picture of the course of the growth in the different cultures. We are first able to state that the productions obtained in test tubes (series A) with 20 and 100 $\mu\text{g P/l}$ agree with those obtained in experiment 982 for these concentrations; the same holds for the curves (omitted from the figures) for 40 and 50 $\mu\text{g P/l}$. Consequently the shaking had no noticeable effect on the growth curve for the conditions existing here.

On the other hand the growth was affected by the repeated changing of the culture solution in the filter tubes (series B). For the lowest concentrations, 10 and 20 $\mu\text{g P/l}$, however, not higher, as one would expect, but on the contrary lower yields were obtained in the filter tubes than in the test tubes. A definite explanation for this cannot be given on the ground of the available data, but it appears probable that the principal cause is to be sought in the differences in the pH of the solutions. In culture solution VIII the pH lies at about 7.0—7.5, but for *Scenedesmus quadricauda* the growth reaches its optimum at about pH 8.0 (cf. ÖSTERLIND 1947). In a limited volume, e. g. 10 ml, the assimilation activity of the algae after only a few days increases the pH to this favourable level, but in the filter tubes, where the solution is changed daily, this is not possible, in any case not with low algal production. If the better phosphate supply in the filter culture is in this way counteracted by a less favourable pH level, the result could be worse growth than in a test tube, provided the phosphate effect is not very great. But whatever the correct explanation may be, it is obvious that for the phosphate concentrations 10 and 20 $\mu\text{g P/l}$, which are nearest to the most usual phosphate contents in nature, the repeated changing of the culture solution could not essentially alter the results obtained

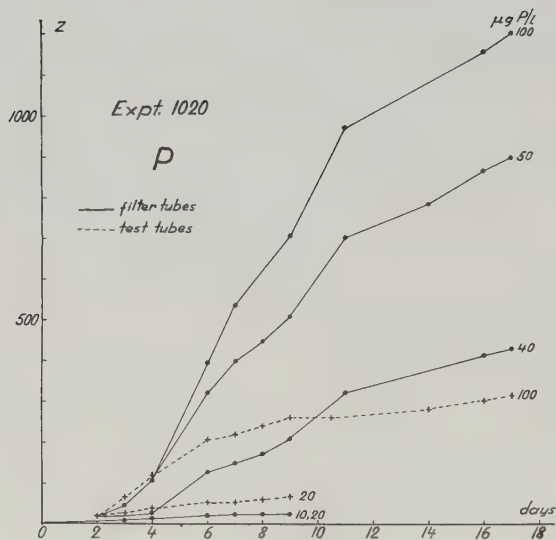


Fig. 13.

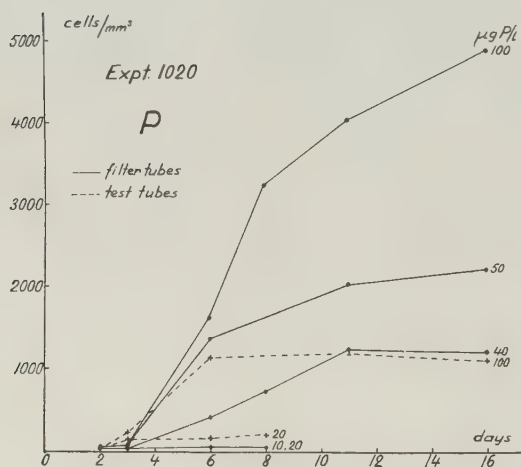


Fig. 14.

Growth (extinction values, Z , fig. 13; and cell numbers, fig. 14) of *Scenedesmus quadricauda* in filter tubes and test tubes for different concentrations of phosphate-phosphorus.

in experiment 982 above. The apprehensions stated above (p. 66) can thus be rejected for these concentrations.

For the higher phosphate concentrations, on the other hand, the repeated renewal of the culture solution caused a considerable increase of production. After 11 days, 40 $\mu\text{g P/l}$ in the filter tube culture gave as high

a yield as 100 $\mu\text{g P/l}$ in a closed volume, and 100 $\mu\text{g P/l}$ in the filter tube gave about as rapid growth as 500 $\mu\text{g P/l}$ in the test tube (expt. 982). This suggests that even fairly large amounts of phosphate are consumed rather rapidly, if the cell concentration is high. For cultures in closed volumes the growth curve is clearly rather a result of the nutrition supply per cell than of the amount of nutrition per unit volume of solution (cf. p. 101).

It is further evident from figs 13 and 14 that even the filter tube cultures — despite their undiminished supply of nutrition — gave growth curves the slopes of which began to decrease after the first 6—10 days. This diminution of the rate of growth can scarcely be explained by the decrease of the supply of light when the production becomes very high; because, for 40 and 50 $\mu\text{g P/l}$, the same diminution of the growth was obtained, despite the densities of these cultures being considerably less than for maximal rate of growth in 100 $\mu\text{g P/l}$. Neither could the pH nor the supply of CO_2 be conceived to give this effect. It can therefore be asked if not growth-inhibiting metabolites within the cells, despite the daily change of the solution, can cause the diminished increase in older cultures. The answering of this question lies, however, outside the frame of the problems treated here.

The dissolved phosphate, as is known, constitutes only the smaller part of the total phosphorus content in the epilimnion of the lakes. JUDAY & BIRGE (1931) state, as the average value for 479 lakes in north-eastern Wisconsin, 3 $\mu\text{g P/l}$ (= 13 % of the total phosphorus content) as dissolved phosphate and 20 $\mu\text{g P/l}$ (= 87 % of the total phosphorus) as organically bound phosphorus in dissolved or solid form. These results have since then been confirmed in principle for lakes in other regions. It can be mentioned here that HUTCHINSON (1941 p. 44) for the surface water in Linsley Pond in Connecticut obtained on the average the value 21 $\mu\text{g P/l}$ for the total phosphorus, of which, however, only 1.7 $\mu\text{g P/l}$ (= 8 %) existed as phosphate. Of the organic phosphorus (92 % of the total phosphorus content), a mean of 13.3 $\mu\text{g P/l}$ was in the solid form ("sestonic phosphorus") and 6.0 $\mu\text{g P/l}$ in solution.

These values give an idea of the extraordinarily small amounts of phosphate which usually stand at the disposal of the plankton algae. They show that the phosphorus generally occurs more richly in organic form than as dissolved phosphate. CHU (1946) has shown recently that some marine diatoms can effectively use phytin and glycerophosphoric acid as sources of phosphorus. In the following experiments I intended to obtain guidance concerning the ability of *Scenedesmus quadricauda* to utilize organic phosphorus.

In order to compare the growth for different additions of phosphorus

with the growth given by the phosphorus content of a lake water, I used surface water from Erken (Sept. 24th, 1945), which was filtered through a bacteria-proof porcelain filter (Schott und Gen., Jena, "17 G 5 auf 3") under sterile conditions. Nitrogen and iron were eliminated as growth limiting factors by the addition of $\text{Ca}(\text{NO}_3)_2$ and ferric citrate — citric acid according to culture solution VIII (= 10.2 mg N/l and 0.18 mg Fe/l).

In the one experiment (802 C) the phosphorus was added as K_2HPO_4 in the concentrations 0, 5, 10, 15, and 20 $\mu\text{g P/l}$, while the other series (803 C) contained 0, 5, 10, and 20 $\mu\text{g P/l}$ as adenylic acid (prepared by Prof. HAMMARSTEN, Stockholm, from yeast nucleic acid; probable formula $\text{C}_{10}\text{H}_{14}\text{O}_7\text{N}_5\text{P} \cdot \text{H}_2\text{O}$, i. e., molecular weight 365 with 1 part of P in 11.4 parts of adenylic acid). The series were started with a few days' interval and carried out under the same experimental conditions: temperature 25° C, light 20 hours per day, continuous shaking. They were inoculated from two parallel cultures of phosphate-starved *Scenedesmus quadricauda* in culture solution VIII(P = 0). The results are given in Table 10.

After subtracting the control values, 10 and 20 $\mu\text{g P/l}$ in 802 C give approximately the same value as those obtained with the corresponding concentrations of phosphorus in expt. 982, where a synthetic solution was used (cf. fig. 10). Erken water thus contains no factor which in any way affects the phosphorus dependence of *Scenedesmus quadricauda*. Further, the assimilable phosphorus content of the lake water itself appears to correspond to nearly 10 $\mu\text{g P/l}$ as K_2HPO_4 . Such a high phosphate concentra-

Table 10. Expts 802 C and 803 C. Growth (extinction values, Z) of *Scenedesmus quadricauda* in Erken water after different additions of inorganic and organic phosphorus respectively.

$\mu\text{g P/l}$	2 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Expt. 802 C. The phosphorus added as K_2HPO_4 .										
0	1	7	17	20	22		27	31	25	16
5	2	10	19	24	26		38	41	47	38
10	12	24	30	36	40		52	57	60	35
15	7	25	36	43	52		62	77	71	59
20	12	31	46	56	64		76	81	78	69
Expt. 803 C. The phosphorus added as adenylic acid.										
0	—8	—2	5	12	13	13	15		15	9
5	—5	11	11	16	11	13	17		21	18
10	—4	23	29	36	35	45	48		50	50
20	—8	25	31	34	37	46	43		49	49

tion is, however, extremely improbable, wherefore even experiment 802 C is in favour of the organic phosphorus being utilizable for the growth of *Scenedesmus*.

This conclusion is confirmed by experiment 803 C, where *Scenedesmus*, however, was not equally willing to start growth (negative values for the first days). The following development shows that the phosphorus bound in adenylic acid can be used for the growth. The yields are somewhat lower than with the corresponding additions of K_2HPO_4 , but this, like the negative values at the start of the experiment, can depend on differences between the two stock cultures which were used for inoculations. Similarly, it can be a coincidence that 20 μg P/l here has not given higher production than 10 μg P/l.

The concentration of dissolved phosphate in numerous natural waters is often so extraordinarily low that one not seldom obtains a completely negative result with the very sensible molybdate method. This means that the phosphate content is less than 1 μg P/l and perhaps simply = 0, which was the case in a good 11 % of the Wisconsin lakes investigated by JUDAY & BIRGE (1931 p. 356). It is notable that such a lack of phosphate exists even in eutrophic waters (especially in the middle of the vegetation period). It is probable that the soluble organic phosphorus in such cases is the main source of phosphorus for the growth of the plankton algae.

As early as 1932 however, FRANZEW (in an important paper which has not won international consideration, probably because it is written in Russian) stated that *Scenedesmus quadricauda* and *Pandorina morum* were capable of continuing growth for a time even if the nitrogen or phosphorus source, or both, were completely removed from the culture solution. The algae could in such a case obviously make use of their own store of nitrogen and phosphorus, and it was proved by analysis (loc. cit., fig. 5 and Tables 3 and 4) that in such starvation-cultures the N- and P-content of the cells were considerably diminished. Thus FRANZEW found that *Scenedesmus quadricauda*, which with plentiful supply of phosphate contained 8.7×10^{-6} μg P/cell, after only three days of growth without phosphate had 9.2×10^{-7} μg P/cell.

KETCHUM (1939) came to a similar result in investigations with the marine diatom *Nitzschia closterium* and with *Chlorella pyrenoidosa*. He found that even after the complete disappearance of the phosphate from the culture solution, the cell division continued with undiminished intensity for some days, whereby the phosphorus content of the cells could decrease to 20 % of the normal. By this, a "phosphorus debt" arose in the cells, which with renewed supply of phosphorus could be covered very quickly (within the course of one day) in dark as well as in light.

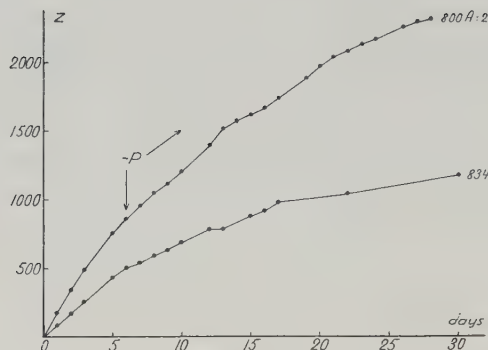


Fig. 15.

Growth (extinction values, Z) of *Scenedesmus quadricauda* in filter tube cultures, before and after the removal of the phosphate. Refer to the text.

In order to obtain phosphate-starved cultures of *Scenedesmus*, I have used the filter tube method described above (p. 67). The course of the substance production for such a starvation experiment (25°C , 20 hours' illumination per day, shaking) is given in fig. 15 by the extinction increase (Z). Culture 800 A: 2 was started (for $e_0 \times 10^3 = 310$) in a complete solution VIII; after six days' rapid increase ($Z = 853$) the medium was replaced with VIII(P = 0) and afterwards both the inner and outer solutions were exchanged for new VIII(P = 0) after each photometric measurement, i. e., practically every day.

The removal of the phosphate ion from the solution obviously had no immediate effect on the substance production; the curve showed the usual progress and after 22 days the culture was still green and highly productive ($Z = 2,298$). It was then divided into equal amounts in six new filter tubes, 834 a—f ($e_0 \times 10^3$ on the average = 700). In these the substance production continued, but after 16 days it became fairly insignificant, as follows from the average curve in fig. 15. At the same time the green of the cultures became obviously pale. 30 days after the start the cultures 834 a—f (Z on the average = 1,168) were used for the experiment (No. 840) described below.

After the removal of the phosphate, the extinction value in 800 A: 2 increased from $310 + 853 = 1,163$ to $310 + 2,298 = 2,608$, i. e., 2.2 times, and in 834 a—f on the average from 700 to $700 + 1,168 = 1,868$, i. e., 2.7 times, or a total of $2.2 \times 2.7 = 6$ times. Since the extinction values do not stand in a linear relation to the amount of substance but with increasing production involve an increasing amount of substance per Z -unit (p. 14), the *Scenedesmus* cultures during the phosphate-free period ($22 + 30 =$

52 days) have increased the initial substance content considerably more than six times. This can scarcely be conceived to have happened, if the cells did not have at their disposal an excess of phosphorus, which could be used when the phosphate was removed from the culture solution. When, after 40—50 days, the substance production had begun to cease, one can assume, with guidance from the results of FRANZEW and KETCHUM, that the phosphorus store of the cells is exhausted and that a considerable phosphorus debt should exist.

The question is now, how great phosphate concentrations and how long time of contact with these are needed to cover the phosphorus deficiency of the cells and restart their growth. KETCHUM found that the phosphate absorption by phosphate deficient cells in the dark was independent of the phosphate concentration in the medium and proportional to the time during which the cells had been grown in the phosphate-free medium (in light there was a further phosphate absorption, proportional to the number of cells present, to provide for photosynthesis). His culture material can not have been so famished of phosphate as the *Scenedesmus* material obtained here by the filter tube method, and in his culture the cessation of the growth probably partly depended on the formation of inhibiting substances. In order to investigate the question further I started the following experiment (840).

From each of the phosphate-starvation filter cultures 834 a—f, 2×0.5 ml of the algal suspension were taken out after shaking; each of these portions was placed in a culture tube with 9.5 ml VIII ($P = 0$) (thus 12 culture tubes in all, designated 840 a: 1 and a: 1', b: 1 and b: 1' . . . f: 1 and f: 1'. The tubes were immediately photometrically measured). After that 834 a—f were washed through with the following culture solutions, replacing VIII ($P = 0$):

834 a	VIII ($P = 0$)	0 μg P/l
834 b	VIII ($P = 5$)	5 » »
834 c	VIII ($P = 10$)	10 » »
834 d	VIII ($P = 20$)	20 » »
834 e	VIII ($P = 100$)	100 » »
834 f	VIII ($P = 200$)	200 » »

The filter tubes were placed as earlier at 25°C , with illumination for 20 hours per day, and continuous shaking; in the same photothermostat were also placed the sub-cultures 840 a—f and a'—f' as well as the sub-cultures started later.

Twenty-four hours later, 834 a—f were washed through twice with VIII ($P = 0$), after which the sub-cultures 840 a: 2 and a: 2' . . . f: 2 and f: 2'

were each inoculated with 0.5 ml of algal suspension in the same way as the previous day. After this withdrawal of 2×0.5 ml of algal suspension, the phosphate graded culture solutions were reintroduced in the cultures 834 b—f.

The same procedure was repeated 2, 3, and 7 days after the start. By this means were obtained the sub-culture series 840 a: 3 and a: 3' . . . f: 3 and f: 3' (2 days), 840 a: 4 and a: 4' . . . f: 4 and f: 4' (3 days) and 840 a: 5 and a: 5' . . . f: 5 and f: 5' (7 days). For each of the six phosphate concentrations (0, 5, 10, 20, 100, and 200 $\mu\text{g P/l}$) there were in this way arranged two parallel series of sub-cultures in which the algae of the different culture tubes had been in contact with the phosphate concentrations in question for 0, 1, 2, 3, and 7 days respectively before their resuspension in VIII ($P = 0$). All of these cultures (60 tubes) were photometrically measured practically daily for two weeks.

In the series 840 a—c and a'—c' all the extinction values showed from the very beginning a continual diminution. This means that not even 7 days' access to 5 or 10 $\mu\text{g P/l}$ could restart the growth of the phosphate-starved cells. The series 840 d and d', and e and e' also gave decreasing extinction values (Z negative) for up to three days' contact with 20 and 100 $\mu\text{g P/l}$ respectively, while 7 days' suspension in these concentrations resulted in slightly positive Z -values (between +1 and +14, no definite difference for both concentrations).

The results for the series 840 f and f', i. e., for the algae which had been in contact with 200 $\mu\text{g P/l}$, were completely otherwise (we disregard here the control samples f: 1 and f: 1' which were taken out from 834 f before the phosphate addition and then gave negative Z -values). The sub-cultures which were taken out after one days' contact with the phosphate showed strongly positive Z -values even from and including the first measurement, and this holds to a yet higher degree for those taken out later. Fig. 16 shows the courses of the extinction curves day by day, counted from the day on which the sub-culture in question was inoculated from 834 f; the curves are recorded on the basis of the average values of the parallels. The initial concentrations were practically equal for f: 2, f: 3, and f: 4, which had $e_0 \times 10^3$ between 130 and 140, while f: 5 had $e_0 \times 10^3 = 163$.

The dashed curves in fig. 16 give the extremes of the growth curves (Z -values) obtained in other experiments — not described here — which were started with small inocula, and where *Scenedesmus* was grown in culture tubes with 10 ml of VIII ($P = 200$) under the same external experimental conditions as for experiment 840 f. After thirteen days the yield for 200 $\mu\text{g P/l}$ (in a closed volume) had varied between the limits $Z = 445$ (798 B: 6) and $Z = 579$ (798 A: 6). In experiment 840 f: 5 the increase

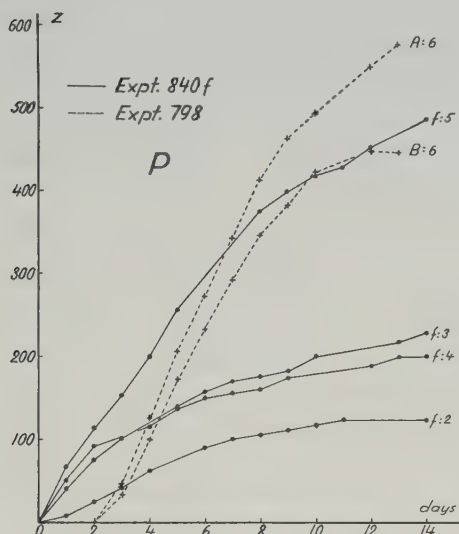


Fig. 16.

Growth (extinction values, Z) of *Scenedesmus quadricauda* in phosphate-free medium (Expt. 840 f) and in a medium initially containing $200 \mu\text{g P/l}$ (Expt. 798). Refer to the text.

of extinction at this time was 470. This shows that the algae, totally starved of phosphate in 834 f, after staying for 7 days in $200 \mu\text{g P/l}$ had not only covered their shortage of phosphate but had even stored so much "extra-phosphate" that the growth after the replacement into the phosphate-free solution could give approximately the same extinction increase as was obtained if *Scenedesmus* was allowed to grow directly in this phosphate concentration. The relative increase was on the other hand less in 840 f: 5 ($e_0 \times 10^3 = 163$) than in both the cultures mentioned ($e_0 \times 10^3 = 38$ and 27). *Scenedesmus* obviously has the ability, on access to phosphate, to accumulate this beyond the immediate need. Compared with the covering of the phosphorus debt, which with sufficiently high phosphate concentration ($200 \mu\text{g P/l}$) obviously went extraordinarily rapidly, this accumulation of extra-phosphate required a fairly long time. It is possible that more than seven days' contact with $200 \mu\text{g P/l}$ would have resulted in a yet higher production than that obtained here.

Concerning *Asterionella formosa*.

The following experiments were made with clone cultures of *Asterionella formosa* HASSALL from the lake Erken. CHU (1942, 1943) has also in-

vestigated the growth of *Asterionella*. His material was isolated from the Barn Elms Reservoir and from Lake Windermere (1942 p. 321). According to the Freshwater Biological Association (F. B. A.), Annual Reports 1946 (p. 28) and 1947 (p. 18), the species in Lake Windermere is *A. formosa* and according to F. J. TAYLOR (private communication) the same is the case for the Barn Elms Reservoir. It seems therefore almost certain that the *Asterionella* cultivated by CHU was *A. formosa* and not *A. gracillima* (HANTZSCH) HEIBERG as he stated.

In his synthetic solutions CHU (1943) obtained no, or very unimportant, growth of *Asterionella* for 9 and 18 $\mu\text{g P(PO}_4\text{)}/\text{l}$. I have had the same experiences. A diminution of the phosphate content of culture solution VIII resulted in decreased yield of *A. formosa*, and for 10 $\mu\text{g P}/\text{l}$ no growth at all was obtained. In consideration of the fact that *Asterionella formosa* is very common even in lakes where the assimilable phosphorus content can scarcely reach 10 $\mu\text{g P}/\text{l}$, I had not expected this. In order to come as near as possible to the natural conditions I started therefore an experiment (No. 838) with bacteria-filtered Erken surface water (taken October 22nd, 1945). Three parallel series (A, B, C), each with 11 culture tubes (10 ml solution), obtained phosphate additions corresponding to 0, 1, 2, 3, 4, 5, 7, 10, 20, 50, and 100 $\mu\text{g P}/\text{l}$. The A-series received no other addition, while the B-series obtained $\text{Ca(NO}_3\text{)}_2$ corresponding to 500 $\mu\text{g N}/\text{l}$ and the C-series contained both this nitrogen addition and ferric citrate—citric acid corresponding to 100 $\mu\text{g Fe}/\text{l}$. An 8-cell *Asterionella* star was then isolated with micro-manipulator into each tube from a clone culture in bacteria-filtered Erken water alone. The tubes were placed in a photothermostat at 15° C and 12 hours' illumination per day.

Two weeks after the start, one could establish with the hand-lens or even the naked eye that in four of the tubes of the A-series and in the same number of the B-series no growth at all had taken place, while the remaining seven tubes in these series had a fairly uneven production. On the other hand all the tubes in the C-series showed a considerable increase which here seemed to be very even except for the tube which contained no phosphate addition and one tube (C: 4) where, at the same time with *Asterionella*, *Chlorella* also grew. From each tube in series C was taken out 1 ml of algal suspension and after suitable dilution (10 to 100 times) the number of cells and stars was counted in a KOLKWITZ chamber of 1 ml. For five of the tubes the counting was repeated about two weeks later (after a total of 31 days). The results, expressed per 10 ml of culture solution, i. e., the whole culture, are shown in Table 11.

It is remarkable that even the resources of assimilable phosphorus in the lake water itself were sufficient for a considerable growth; in C: 1 the

Table 11. Expt. 838. Growth of *Asterionella formosa* in Erken water with different additions of K_2HPO_4 . Inoculum: 8 cells (1 star).

838	Addition μg P/l	After thirteen days			After thirty-one days		
		<i>a</i> cells	<i>b</i> stars	<i>a/b</i>	<i>a</i> cells	<i>b</i> stars	<i>a/b</i>
C: 1	0	1,270	165	7.7	1,208	340	3.6
C: 2	1	53,000	8,620	6.1	228,000	169,500	1.3
C: 3	2	161,000	49,800	3.2	308,000	203,900	1.5
C: 4 ¹	3	41,700	16,010	2.6			
C: 5	4	138,000	47,900	2.9			
C: 6	5	122,500	36,300	3.4			
C: 7	7	182,000	43,000	4.2	238,000	164,100	1.4
C: 8	10	139,000	42,400	3.3			
C: 9	20	101,500	44,050	2.3			
C: 10	50	54,300	25,060	2.2			
C: 11	100	95,000	31,050	3.1	233,000	179,100	1.3
B: 1	0	12,500	2,500	5.0			

¹ With *Chlorella* in good development.

inoculated eight cells increased in a fortnight to 1,270 cells, whilst the multiplication in B: 1 gave 12,500. An addition of only 1 μg P/l (C: 2) had a very great effect, giving a nearly 200 times higher final yield than in C: 1, and for an addition of not more than 2 μg P/l both the highest rate of growth and the greatest final production were obtained (C: 3). Additions of 50—100 μg P/l had an inhibiting effect on the rate of growth but apparently not on the final level (C: 10, C: 11).

This phosphate dependence has no resemblance to that reported by CHU (1943 fig. 21) or with my own experiences from cultures in medium VIII and other synthetic solutions. *Asterionella* can clearly utilize very small increases of phosphate much better if grown in lake water than in solutions with inorganic salts alone (+ citrate and citric acid in negligible amounts). It appears, therefore, as if lake water would contain one or several factors which principally change the phosphate dependence of *Asterionella* and facilitate its use of low phosphate concentrations. This assumption is confirmed by similar results at the fresh-water biological station Wray Castle at Lake Windermere (F. B. A., Annual Reports 1945 p. 20, and 1946 p. 26).

During the summer and autumn of 1946, at the Erken laboratory, I tried in numerous series of experiments, which to a large extent agreed in their arrangement with the experiment 838 described, to grasp the reason why *Asterionella formosa* in lake waters can make use of phosphate concentrations which in synthetic solution had no effect whatever on the

growth. The experimental results gave very little. The result obtained for experiment 838 was certainly in principle confirmed, but on the other hand no positive conclusions could be obtained from the new experiments; for this reason I refrain from giving a closer account of them. The cause itself of this failure nevertheless deserves mentioning.

For these experiments, instead of the usual culture tubes, I used tubes of about the same dimensions but with a plane bottom. With the help of an inverted microscope (according to UTERMÖHL) objects on the bottom could thereby be directly studied and the growth of, e. g., an *Asterionella* star could in this way be comfortably followed from day to day without any interference of the culture. When the production became too high for direct counting or if the *Asterionella* stars floated in the solution (which often characterized the best periods of the growth), the counts were carried out instead in the usual way in a KOLKWITZ or a BÜRGER chamber after extraction of a small quantity of the algal suspension.

For inoculation of each experiment was usually used a specially prepared clone culture, originating from an *Asterionella* star which, from a newly taken Erken sample, had been isolated direct to bacteria-filtered Erken water. In preparing these stock cultures I often had the experience that the multiplication ability of *Asterionella* varied considerably from case to case. The same observations have been made at Wray Castle concerning *Asterionella* in Windermere (F. B. A., Annual Report 1943 p. 18), where, particularly after the production of a maximum in the lake, a kind of "physiological dormancy" could be established when *Asterionella* colonies were taken in culture. The cause of this varying activity, however, has hitherto not been clarified.

Asterionella appeared yet more mysterious and erratic in many of my series of experiments, despite the different tubes in each experiment being inoculated with a single star each from the same clone culture. Even identical simultaneous cultures could give quite different increases (cf. Table 11, 838 B: 1 and C: 1, where B: 1 in its qualifications only differs from C: 1 by the lack of addition of iron). As an example can be mentioned that on one occasion in a culture without phosphate addition (but with Fe and N, thus = 838 C: 1) I obtained after 30 days 240,000 cells (!), while the one parallel culture gave an "ordinary" increase to 1,240 cells; in the other parallel sample on the other hand only a doubling of the cell number (from 8 to 16) took place! When even a material homogeneous in origin and treatment could give rise to such a divergent increase it follows that it was scarcely possible to bring about reproducible results. This is the reason why I cannot draw any definite conclusions from my *Asterionella* experiments in the summer and autumn of 1946.

According to both my own experiences and those announced from Wray Castle the phosphate dependence of the growth of *Asterionella* is consequently of a very complicated nature. The phosphate concentration is not decisive in itself but its utilization is to a high degree affected by one or several factors existing in lake water. Further, the activity of the cells is, for an unknown cause, particularly variable. The equation therefore still contains too many unknowns to be able to be solved.

We can state, however, that the phosphate concentrations existing in the lakes are sufficient to give rise to a rich development of *Asterionella* under favourable conditions. In this respect *Asterionella formosa* parts in point of principle from *Scenedesmus quadricauda*, and this difference can be one of the causes why *Asterionella* in contrast to *S. quadricauda* often reaches rather high population densities in the lakes.

One asks, however, if the difference to an essential degree cannot consist of an *Asterionella* cell needing considerably less phosphorus for its development than a *Scenedesmus* cell. In order to get an idea of this I have, on the basis of particulars in Tables 9 and 11, made an estimation of the phosphate need per cell for such phosphate concentrations, where only or mainly the phosphorus factor seems to have been decisive for the cell number obtained at the end of the experiment (from this number is subtracted the cell number which at the same time existed in the control culture). The result of this calculation follows from Table 12.

Table 12. Calculation of the phosphate need per cell according to experiments 982 and 838. (Cell increase = final cell concentration — cell concentration of the control culture.)

A. *Scenedesmus quadricauda* (expt. 982; see Table 9).

$\mu\text{g P/culture}$	Cell increase/culture	$\mu\text{g P/cell produced}$
0.1	60×10^4	1.7×10^{-7}
0.2	107×10^4	1.9×10^{-7}
0.3	270×10^4	1.1×10^{-7}
0.4	308×10^4	1.3×10^{-7}
0.5	465×10^4	1.1×10^{-7}
Average:		1.4×10^{-7}

B. *Asterionella formosa* (expt. 838; see Table 11).

$\mu\text{g P added per culture}$	Cell increase/culture	$\mu\text{g P/cell produced}$
0.01	22.7×10^4	4.4×10^{-8}
0.02	30.7×10^4	6.5×10^{-8}
Average:		5.5×10^{-8}

It should now first be expressly pointed out that these values, which are based on a primary material obtained under certain experimental conditions, cannot without further testing be considered to have general validity. Concerning *Scenedesmus quadricauda* it appears probable, however, that the average value obtained in its order of magnitude (10^{-7} $\mu\text{g P/cell}$) can prove to be true even under natural conditions.

With *Asterionella*, on the other hand, it was not possible to obtain reproducible values, because the phosphate dependence of the alga is affected by, up to now, uncontrollable circumstances. These unknown factors are certainly active in the lakes, wherefore the phosphate dependence of the alga in nature is possibly very variable. Since the yields obtained in experiment 838 are to a large extent the highest which I have obtained under the experimental conditions in question, 5.5×10^{-8} $\mu\text{g P/cell}$ represents the lowest value which I can give for the phosphate need of *Asterionella*. In other experimental series the best yields were not seldom 10—100 times less than for the corresponding concentration in experiment 838, which means a 10—100 times larger value for the phosphate need per cell (5.5×10^{-7} — 5.5×10^{-6} $\mu\text{g P/cell}$). For comparison it can be mentioned that EINSELE & GRIM (1938) give 60×10^{-6} $\mu\text{g Si}$ as the average value for the silicon content in one cell of *Asterionella* and at the same time give the quotient $\text{Si} : \text{P} = 60 : 1$. (According to Metropolitan Water Board 1939 p. 47, the quotient $\text{Si} : \text{P}$ for *Asterionella formosa* should be about 130 : 1.) An average phosphorus content of about 1×10^{-6} $\mu\text{g P/cell}$ follows from this (cf. the values of FRANZEW quoted above, p. 73, for *Scenedesmus quadricauda*), i. e., a twenty times higher value than the amount of phosphorus which each cell produced in experiments 838 C: 2 and C: 3 had at its disposal. Consequently it appears as if even *Asterionella* under certain conditions could multiply by making use of the phosphorus store of the cell itself.

It appears from what has been said that it is not possible, on the basis of the material available now, to derive a significative relation between the phosphate need of *Asterionella* and that of *Scenedesmus*. Considering that a need of 5.5×10^{-8} $\mu\text{g P/cell}$ should be a minimum value for *Asterionella* and that the normal phosphorus contents of the cells of *Asterionella* and *Scenedesmus* are probably of about the same order of magnitude (10^{-7} — 10^{-6} $\mu\text{g P/cell}$), it is, however, scarcely probable that the average phosphorus need per cell is essentially different for *Asterionella formosa* and *Scenedesmus quadricauda*. The considerable difference in the phosphate dependence of their growths in lake water must therefore depend above all on the ability of *Asterionella* (with the help of a factor existing in the lake water) to make use of even very small concentrations of phosphate in a considerably more effective way than *Scenedesmus*.

Concerning *Dinobryon divergens* and *Uroglena americana*.

In the summer of 1946 I carried out at the Erken laboratory several experiments, with a view to obtaining an idea concerning the environmental requirements of certain difficult to cultivate plankton algae. Since I had not earlier been successful in cultivating these algae in purely synthetic solutions, I started direct from newly taken lake water which was filtered through a paper filter or bacteria-proof porcelain filter. Some observations concerning the phosphate dependence of *Dinobryon divergens* IMHOF and *Uroglena americana* CALKINS are of interest in this present connection, despite the fact that they are only preliminary in nature and must be completed with more detailed investigations.

It proved in these studies that both *Dinobryon* and *Uroglena* are very sensitive to the quality of the glass used and its previous treatment. In a hanging drop on a slide or cover-glass of usual commercial ware or in a test tube of ordinary quality, the algae were destroyed within 5—10 hours: the protoplasts of *Dinobryon* were thrown out of their envelopes and the *Uroglena* colonies were dissociated. After boiling the glass for two hours, the algae usually managed to live a few more hours, but after twenty-four hours they also perished. In new culture tubes of Jena Geräte or Duran glass, they could possibly live for twenty-four hours, but seldom more, but if the tube was boiled, death did not take place before some days later. The best results were obtained with culture tubes of glass of these qualities, which had been in use for a long time; in these both *Dinobryon* and *Uroglena* could be kept alive in Erken water for at least two weeks and even show a fairly good increase.

For my experiments I usually used Duran glass tubes with plane bottoms, which could be studied from underneath with an inverted microscope. The algae were taken direct from a fresh Erken sample or from a stock culture in Erken water and each tube of the series was inoculated with a colony of about the same size. The tubes were placed on a north aspect window table. The temperature, which could not be regulated, was between 20° C and 25° C.

The growth of these algae could of course not be registered by cell counts. The number of colonies were counted instead, and observations were noted in the records concerning the size and condition of the colonies. A detailed reproduction of these partly comprehensive records is scarcely necessary here, but a short summary of the results concerning the phosphate dependence should be sufficient.

In different experimental series K_2HPO_4 , $Ca(NO_3)_2$, and ferric citrate—citric acid were added to bacteria-filtered Erken water. It proved that

additions of up to 100 $\mu\text{g N/l}$ and about 175 $\mu\text{g Fe/l}$ had a fairly unimportant effect on the growth of *Dinobryon* and *Uroglena*. With phosphate, on the other hand, quite unexpected effects were recorded. For additions of 0.5—4 $\mu\text{g P/l}$ increased growth was sometimes obtained, but on many occasions no difference in relation to the controls (in Erken water only) could be observed for these concentrations. Additions of 5—10 $\mu\text{g P/l}$ or more gave, however, definite negative effects. At first I did not trust this result, but with repeated experiments I always obtained the same progress: while the inoculated colony of *Dinobryon* or *Uroglena* multiplied to 20—50 colonies in about two weeks if grown in Erken water only, or in water with less than 5 $\mu\text{g P/l}$ added, the growth was inhibited by even slightly higher additions of phosphate. This negative phosphate effect was very obvious, because the *Dinobryon* cells evacuated their envelopes and the *Uroglena* colonies completely disintegrated, being more rapid the higher the phosphate addition (usually within 3—6 days for 5—10 $\mu\text{g P/l}$).

At the same time as these studies, I tried to get *Gloeotrichia echinulata* (J. E. SMITH) RICHTER to increase in culture. As mentioned above (p. 62) I was not successful with this before I (at a later date) added fresh garden soil to Erken water. Therefore I could not arrange any continuous experimental series, but on several occasions I received the impression that the degeneration of the *Gloeotrichia* globules was accelerated by addition of phosphate, even if the concentration of added phosphate reached only about 10 $\mu\text{g P/l}$. If this observation should be confirmed by experiments in which controls with *Gloeotrichia* in good and uninterrupted growth are available, the phosphate dependence of *Gloeotrichia* should be of about the same type as that of *Dinobryon* and *Uroglena*.

It seems justified here to speak of a special type of phosphate dependence. The phosphate supply of the water of Erken for these algae seems to be within or immediately below their optimum interval, the upper limit of which lies so low that an increase of the phosphate concentration by only about 5 $\mu\text{g P/l}$ has an inhibiting effect on the growth or directly damages the algae. We remind ourselves that it required 500—1,000 $\mu\text{g P/l}$ in order to reach the lower limit of the optimum interval for *Scenedesmus quadricauda* and additions of 2—20 $\mu\text{g P/l}$ to obtain maximum rate of growth of *Asterionella formosa* in Erken water.

The results obtained from these culture experiments in Erken water, as I have already pointed out, are to be regarded as preliminary. They must be retested under completely controllable conditions, i. e., in solutions with exactly known composition. However they plainly point in the direction that the supply of phosphorus in the lakes may not generally be regarded as a minimum factor; for certain plankton algae it can apparently

be within the optimum or simply, by a proportionally unimportant increase, appear as a maximum factor, inhibiting or perhaps also preventing growth of these algae.

Dinobryon divergens and *Uroglena americana* are very common in both humic-oligotrophic and eutrophic lakes. It is, however, conspicuous that they usually do not exist in lakes which are exposed to enrichment of the nutrients (especially phosphorus and nitrogen) by human habitation. Thus the genus *Dinobryon*, according to THUNMARK (1945 a), is completely lacking in the polluted lakes Väcksjön (about 100 μg total phosphorus/l, ÅBERG & RODHE 1942 p. 158), Trummen, and Södra Bergundasjön. The lake Norrviken (15 km north of Stockholm) has in a similar way been strongly eutrophicated (total phosphorus content 100—200 μg /l) by industrial and domestic drainage; according to investigations of H. SKUJA and the author during 1946 and 1947, *Uroglena* is completely lacking in this lake, while isolated colonies of *Dinobryon divergens* have been observed only three times (in winter and spring 1947). It would be precipitate to ascribe this effect to the phosphorus factor alone, but if the results obtained above should prove to be correct, the high phosphorus concentration must already be sufficient reason for *Dinobryon* and *Uroglena* avoiding polluted waters.

Concerning the periodicity and the regional distribution of *Dinobryon divergens*, PEARSALL (1932) considered that he could establish a relation partly with the silicon content (increasing production for decreasing Si-concentration) and partly with the quotient nitrate-nitrogen/phosphate-phosphorus (favourable effect of a high ratio, unfavourable of a low). HUTCHINSON (1944) has taken up this hypothesis in a thorough examination and has thereby, on the ground of personal experiences and available data in the literature, arrived at the conclusion that direct relations of that kind, as PEARSALL assumed, may not exist. HUTCHINSON could not, however, discover any other immediate correlation with the chemical factors, but he made the observation (p. 22) that "the only circumstance common to all the cases that have been reported appears to be that the rise in *Dinobryon* occurs after a rapid decline of large populations of other species, which may be diatoms, *Scenedesmus* or blue-green algae. . . . When the latter have exhausted the nutrients to a point at which the population cannot continue to expand, the inevitable decline heralds the rise of *Dinobryon*. While it is clear that *Dinobryon* can increase at lower nutrient levels than can the antecedent species, the mechanism of its suppression at higher levels is entirely obscure: the problem is, however, advanced slightly by recognizing that the incidence of the *Dinobryon* maximum is probably determined by biotic rather than physico-chemical factors."

HUTCHINSON realized his discussion of the periodicity of *Dinobryon* and its causes on the basis of the conception that "the freshwater plankton as a whole must be living dangerously close to the development of nutrient debts" (p. 21), not the least as regards the phosphate. If one applies instead the experimental result related above, that by an insignificant increase the phosphate content can become superoptimal and inhibiting to the growth of *Dinobryon*, the relations in Linsley Pond treated by HUTCHINSON appear in a new light. During 1938 "*Dinobryon* was present in very small numbers (< 10 cells per ml) from Jan. 17 to May 4 (4 cells per ml), but in the succeeding week increased rapidly, so that 218 cells per ml were present on May 11, and 688 on May 18, on which date this organism was the dominant determinable species of phytoplankton" (loc. cit., p. 7, see also fig. 2). The phosphate content in the surface water of Linsley Pond according to RILEY (1940 p. 295, fig. 8) was about $4 \mu\text{g P/l}$ during the early spring months, but during the month of May it was only about $1 \mu\text{g P/l}$. The growth of *Dinobryon* and the concentration of phosphate thus give curves of opposite shape. This agrees completely with my experimental results. It seems therefore not to be a coincidence that the correlation between the production of *Dinobryon* and the changes of phosphate content in Linsley Pond had the negative sign. On the other hand the quotient $\text{N}(\text{NO}_3)/\text{P}(\text{PO}_4)$ may not have any significance for *Dinobryon*, and the cause for the maximum of *Dinobryon* following immediately after earlier large populations of *Oscillatoria* and *Scenedesmus* need not be biotic in character. By their phosphate consumption these algae forced down the phosphate concentration to such a low level that, in my opinion, it came below the upper optimum limit for *Dinobryon*.

TRESSLER & DOMOGALLA (1931) have studied the environmental relations and the plankton production in the small Lake Wingra in the vicinity of Madison, Wisconsin. It is evident from their production diagrams (pp. 347 and 349) that *Dinobryon* (*divergens*?) during 1928 existed relatively richly in the beginning of February and in subdominant amounts during May and June, but that it was completely lacking in March and the beginning of April. Just during this intervening time the curve for phosphate-phosphorus (loc. cit., fig. 6; the ordinate values by mistake 10 times too great) shows a maximum with the highest value at about $25 \mu\text{g P/l}$ on March 1st. It no longer surprises us that *Dinobryon* then disappeared, to reappear and reach high production at lower concentrations. TRESSLER & DOMOGALLA were in this case correct when they pointed out (p. 343) that "there are so many conflicting results that no case can be made for phosphorus as a limiting factor in plankton growth in Lake Wingra." On the other hand, *Synedra* appreciated and utilized the better supply of

phosphate to a high degree: an immense production of this diatom (species?) was developed during March and April (loc. cit., p. 349).

A part of my own field investigations has yielded material which strengthens the idea set out above concerning the role of the phosphate content on the periodicity of *Dinobryon divergens*. From a forthcoming publication on environmental conditions and phytoplankton in Uppland lakes I can in this respect give some data concerning phosphorus and nitrogen stratification in the small lake Skärsjön 20 km east of Uppsala (Table 13). It is only 8.2 m deep but, owing to its wind-sheltered situation between well-wooded and partly high shores, it has a rather stable summer stratification. This forms, among other things, a phosphate stratification which with regard to the small depth lacks any correspondence in the limnological literature, so far as is known to me. JUDAY & BIRGE (1931 p. 363) have found somewhat higher values in the 20 m deep Lake Mary but the cause was there partly to be sought in an incomplete spring circulation.

Table 13. Phosphorus and nitrogen concentrations ($\mu\text{g/l}$) in Skärsjön 1941.

P_t = total phosphorus, P_p = phosphate-phosphorus,
 N_t = total nitrogen, N_n = nitrate-nitrogen.

Depth m	May 30th				July 9th				August 21st ¹			
	P_t	P_p	N_t	N_n	P_t	P_p	N_t	N_n	P_t	P_p	N_t	N_n
0	18	1	80	20	19	6	115	27	21	4	90	25
4	25	—	77	23	—	—	—	—	—	—	—	—
5	—	—	—	—	19	—	160	53	—	—	—	—
6	—	—	—	—	—	—	—	—	43	—	150	15
6.5	—	—	—	—	—	—	—	—	107	—	—	—
7.5	43	20	90	25	250	175	340	55	626	470	1,070	25

¹ $\text{N}(\text{NH}_4)$ August 21st: 0 m 0 $\mu\text{g/l}$
 6 m 200 $\mu\text{g/l}$
 7.5 m 500 $\mu\text{g/l}$ } approximate values after direct addition
 of NESSLER's reagent.

In the middle of June 1941, as is often the case at this time (cf. ÅBERG & RODHE 1942 p. 105), chilly weather prevailed with fairly strong winds. In the wind-exposed Erken an intermediate and complete circulation thereby appeared, but in the more sheltered Skärsjön only a partial circulation arose which, judging from the temperature stratification (fig. 17), had a definite effect down to 6 m depth. Thus the upper layer must have been mixed with phosphate-rich hypolimnion water. For the test on July 9th the stratification was found to be stabilized again, but an after-effect

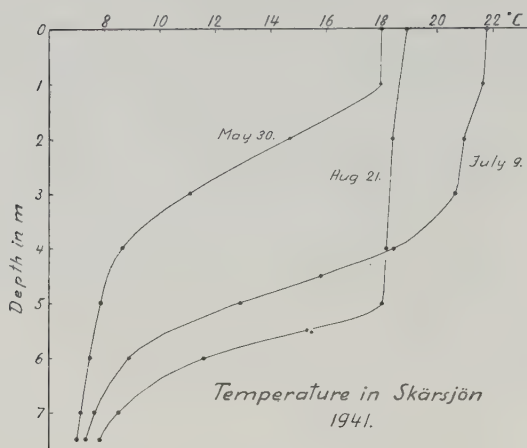


Fig. 17.

Temperature stratification of Skärsjön in summer 1941.

from the partial circulation could still be traced since the phosphate content of the surface water was higher than for the test of May 30th.

SKUJA, from a taxonomic point of view, has studied my phytoplankton material from the lakes of Uppland (SKUJA 1948). He has thereby also tried to estimate the frequencies of the different species. Even if such a subjective method can never give exact values one can in this way obtain an approximate conception of at least the relative proportions of amount. The following notations have been used:

.	:	:	—	=	≡	≡	≡	≡	≡
isolated numerous masses									

The following has been taken out of the tables for Skärsjön (not yet published):

	May 30th	July 9th	August 21st
<i>Dinobryon divergens</i>	≡ ≡ ≡	:	≡ ≡ ≡
<i>Asterionella formosa</i>	.	≡ ≡ ≡	:

After the long and severe winter, Skärsjön was frozen in the year 1941 until May 5th. It is therefore scarcely probable that the immense occurrence of *Dinobryon* on May 30th had been able to be preceded by any other high production, particularly as a net sample from April 16th showed a very

poor phytoplankton production. On the other hand, at the beginning of July, *Dinobryon* for a short time had given way to a high production of *Asterionella*. On August 21st *Dinobryon* was again dominant. For a test on October 31st the relation was, however, again altered: *Asterionella* in high production, *Dinobryon* with the frequency :.

Against the background of my results on the phosphate dependence of these algae this succession becomes fully explicable. Concerning phosphate, *Dinobryon* lives in Skärsjön by no means "dangerously close the development of nutrient debts," on the contrary, a few metres below the surface layer are formed dangerously high phosphate concentrations. It only needs chilly and windy weather to bring about a partial circulation which transports a part of this phosphate to the epilimnion for the growth of *Dinobryon* to be inhibited. One must almost assume that a part of the mass occurrence on May 30th was destroyed in order to understand the considerable diminution to July 9th. On the other hand such a phosphate increase favours the increase of *Asterionella*, which only occurred rarely in the plankton sample of May 30th. When *Asterionella* and other algae had consumed sufficient phosphate, *Dinobryon* could again reach a high production.

According to PEARSALL's hypothesis the ratio between nitrate-nitrogen and phosphate-phosphorus should have conditioned the changes in the production of *Dinobryon* in Skärsjön. It follows, however, from Table 13 that it is mainly the variation of the phosphate which decides the value of the quotient for the three dates. It is extremely improbable that the quotient had any importance for the growth in this case.

The supposition of HUTCHINSON that "biotic rather than physico-chemical factors" should be decisive for the periodicity of *Dinobryon* also appears here to be improbable. Of course one must always allot the biotic factors a very great importance, but at least in this case the course of growth seems, in its principal features, to be conditioned by a single chemical factor, namely, the phosphate concentration. The succession of *Asterionella* can also presumably be here assigned to this factor which then for this alga had a directly opposite effect to that for *Dinobryon*.

Discussion.

The experiments described above have shown that plankton algae can have very different phosphate requirements. One and the same phosphate concentration can for certain species be suboptimal, for others optimal and for some species simply superoptimal for the growth. This must be of great importance for the temporal and regional changes of the species. The ex-

perimental results are confirmed indeed by the observations quoted concerning the ecology of the investigated algae.

In the epilimnion of most lakes, the content of phosphate and of other forms of assimilable phosphorus varies within the interval below 20 $\mu\text{g P/l}$. With regard to their phosphorus needs, the plankton algae can therefore be suitably assigned to the three following categories:

	Optimum	
	lower limit	upper limit
	$\mu\text{g P/l}$	$\mu\text{g P/l}$
I. Low phosphorus requirement.....	< 20	< 20
II. Medium phosphorus requirement....	< 20	> 20
III. High phosphorus requirement.....	> 20	> 20

This scheme is reminiscent of UTERMÖHL'S (1925 p. 360) attempt to fix the "ökologische Teilspektra" of the plankton algae and the similarity is not accidental. But UTERMÖHL set out from ecological observations only and could not therefore quantitatively establish the importance of the individual factors for the growth of the different algae. Neither could he, of course, distinguish with certainty between the limiting effect of a sub-optimal factor and the inhibiting effect of a superoptimal factor.

Of the four plankton algae investigated here, *Dinobryon divergens* and *Uroglena americana* (Chrysophyceae) represent the first, *Asterionella formosa* (Diatomaceae) the second, and *Scenedesmus quadricauda* (Chlorococcales) the third category. According to some of my preliminary experimental observations, the distribution of these algae in the three categories is to a certain degree representative of many other species within the corresponding taxonomic groups. Thus the Chlorococcales usually seem to be relatively needy of phosphorus, while the Chrysophyceae perhaps often belong to category I. The diatoms probably take up an intermediate position which is, however, to be complicated if their phosphorus dependence is affected by other factors in the same way as with *Asterionella*.

In many lakes the main mass of phytoplankton is composed of algae which presumably belong to categories II and III. The established opinion of the importance of the phosphorus content as a limiting factor for the phytoplankton production as a whole is therefore substantially correct, if, in agreement with THIENEMANN (1941 p. 98), one defines this production in a simple quantitative manner as the total amount of phytoplankton (and their excretions) formed during a certain time in the biotope. On the other hand it is wrong to regard, in general, the phosphorus supply as a minimum factor for growth of all plankton algae. A given change of the content of assimilable phosphorus has different effects on the growth of

algae belonging to the categories I, II, and III. An increase of the phosphate content can thus, for algae in group I, bring about diminished growth, while the algae in group III are favoured, and those in category II are not at all affected by this alteration. The temporal and regional changes of the assimilable phosphorus content should thus have not only quantitative but also qualitative effects on the phytoplankton production of the lakes.

Many of our experiences from nature are in favour of this idea, although the simultaneous effect of other factors present often makes it impossible to distinguish with certainty the causal connections. As an example can here be mentioned the great difference in the composition of phytoplankton of oligotrophic and eutrophic lakes (TEILING 1916) and the displacement in the relation between the number of species of Desmidiaceae and Chlorococcales with increasing trophic level (PEARSALL 1932, THUNMARK 1945 a and b). It is possible that such changes of the composition of the phytoplankton, which run their course parallel with a quantitative increase of the standing crop, are partly conditioned by principal differences in the phosphorus dependences of the plankton algae. However, only continued experimental investigations can give a reliable answer on this point.

It appears from what has been said, that for the treatment of the phytoplankton production and its conditions, one must coordinate the quantitative aspect with the qualitative. None of them alone gives a correct picture of the real facts. It is further necessary to learn to know the physiology and ecology of the individual species, since the total production is only a summation of the development of the different components. This, in its turn, is based on the prevailing environmental conditions, the proper study of which is therefore of great importance in this connection also. Concerning the supply of phosphorus, particularly great difficulties are met here, since there is still no method to determine quantitatively the content of assimilable phosphorus. This content, which is perhaps of different significance for different algae, should, with pure cultures of plankton algae, be establishable in a biological way. The work carried out hitherto to this end (see, e. g., SCHREIBER 1927, FRANZEW 1932, MÜNSTER STRØM 1933, GUSSEVA 1938) has not led to a definite result. My own experiments have been unsuccessful owing to the difficulty of finding a test-object which is sufficiently sensitive and gives reproducible evidence which is easy to record.

VI. Influence of Nitrate Deficiency on Growth and Chlorophyll Formation.

Introduction.

Along with phosphorus, nitrogen is considered to be the most important limiting factor for the development of phytoplankton. Ammonium and nitrate first come in question as inorganic sources of nitrogen, and of these the nitrate ions occur most abundantly in the surface layers of natural waters. DEEVEY (1941 p. 728) obtained the correlation coefficient $+0.605$ for the connection between the contents of chlorophyll (in summer) and nitrate (in winter) in a number of lakes in Connecticut, consequently a lower coefficient than for the connection of chlorophyll with total phosphorus ($+0.919$, cf. above, p. 64). The highest coefficient, $+0.964$, was obtained, however, by comparing the chlorophyll content at the same time with both the nitrate content and the phosphorus content. Other results could also be quoted from the limnological literature to show that nitrogen and phosphorus are interfering as limiting factors in the total production of phytoplankton.

CHU (1943) has experimentally investigated the effect of different nitrogen concentrations on the growth of some plankton algae. He found (p. 146) that "the lower limit of the optimum range of nitrogen concentration differs for different organisms. It generally varies approximately from 0.3 (*Fragilaria crotonensis*) to 1.3 (*Pediastrum boryanum*, *Nitzschia palea*) and sometimes to 2.6 or 5.3 (*Staurastrum paradoxum*) p. p. m., when an ammonium salt is the source of nitrogen; and from 0.3 (*Botryococcus Braunii*) to 0.9 (*Staurastrum paradoxum*) p. p. m. when nitrate is the source of nitrogen." These values are higher than the content of nitrate- and ammonium-nitrogen in the trophogenic layers of most natural waters.

Nitrogen belongs to the nutritive substances which are necessary for the formation of chlorophyll (RABINOWITCH 1945 p. 428). In the case of nitrogen deficiency, green plants become chlorotic, and CHU (loc. cit., p. 147) found that "the nitrogen concentration determines to a large extent the amount of chlorophyll formed" in pure cultures of plankton algae. In

the following experiments I have carried out chlorophyll determinations in parallel with determinations of the extinction and the cell concentration of the cultures.

Experiments with *Scenedesmus quadricauda*.

In an experiment described earlier (No. 913, see p. 19) it was established that the chlorophyll formation of *Scenedesmus quadricauda* is more sensitive than the substance production and the cell increase to a deficiency of nitrogen. This appears particularly obvious if the source of nitrogen is suddenly and completely removed from the medium of a highly productive and beautifully green *Scenedesmus* culture. Such nitrate-starved cultures can easily be obtained by filter tubes (p. 68, fig. 12). As culture solution I have used medium VIII without nitrate, VIII (N = 0), the calcium nitrate being replaced by an equivalent amount of CaCl_2 . The culture solution was often changed, preferably every day. After only two days' absence of nitrate the cultures began to pale, and after two to three weeks they were usually completely colourless. Despite this, the substance production and cell increase continued for a further few weeks, before they gradually ceased. A similar observation was made by PIRSON (1937 p. 243) on nitrate deficient cultures of *Chlorella*.

It is obvious that the nitrogen store of the cells must be used up for this continued growth in nitrogen-free medium. According to FRANZEW (1932 Tables 3 and 4), three days' nitrogen starvation of *Scenedesmus quadricauda* was accompanied by a decrease of the nitrogen content of the cells from 81×10^{-6} to 14×10^{-6} μg N/cell.

Ageing highly productive cultures in solution VIII usually show the same external symptoms as have just been described, and this, at least partly, for the same reason: lack of nitrogen. Their nitrogen starvation cannot, however, go so far as in a filter tube culture, while lack of other nutrients and probably also formation of inhibiting substances previously force the increase to cease.

In order to compare the dependence on nitrogen of *Scenedesmus* from an ageing and from a nitrogen-starved culture, a stock culture of each kind was prepared. The one (927 A) grew in a flask with a complete culture solution VIII, while the other (896 A) was carried out in a filter tube with culture solution VIII (N = 0), repeatedly changed. The extinction values of the stock cultures and their chlorophyll content during the weeks before the start of the main experiment are shown in Table 14.

Table 14. Extinction ($e \times 10^3$) and chlorophyll content ($\mu\text{g/ml}$) of the stock cultures 927 A and 896 A during the time just before the start of the main experiment (929).

Date	927 A		896 A	
	Extinction	Chlorophyll	Extinction	Chlorophyll
March 26	150	5.2	129	2.9
» 27	181	7.6	167	3.8
» 28	233	9.5	185	3.7
» 29	281	11.3	205	3.2
» 30	331	12.3	212	2.8
April 1	449	12.4	223	2.6

On April 1st the formation of chlorophyll had obviously practically ceased in the ageing culture 927 A, while a diminution of the chlorophyll took place in the nitrate-starved culture 896 A after March 27th. The substance production, on the other hand, continued in both cultures, but considerably more quickly in 927 A than in the filter tube culture.

On the day mentioned, the series of experiments 929 A and 929 B were started in culture solution VIII ($N = 0$) with different additions of nitrate. Culture 927 A was filtered with a glass filter and the algae left on the filter were washed with VIII ($N = 0$) and suspended in 500 ml of this solution. The algal suspension was divided into 10 flasks (929 A: 1—10) with 50 ml in each. 896 A was treated in the same way, being divided into the flasks 929 B: 1—10. The nitrate was graded from 0.0 to 5.0 mg N/l by different additions of $\text{Ca}(\text{NO}_3)_2$. An extract from the experimental record is given in Table 15.

It clearly follows from the values of both series that a nitrate concentration of 0.5 mg N/l or less is strongly suboptimal for both the growth and the formation of chlorophyll with *Scenedesmus quadricauda*. On the other hand 5.0 mg $\text{N}(\text{NO}_3)/\text{l}$ generally gave a marked increase, but we observe that not even this nitrate content, which is very high in relation to the nitrate concentrations in the surface layer of natural waters, was sufficient for more than two days' chlorophyll formation. The substance production, which, like the cell increase, went on even in cultures without nitrate, continued, on the other hand, without apparent diminution after the cessation of the increase of chlorophyll.

The values in Table 15 are not directly suitable for a comparison of the results of the two series, since their initial concentrations were different. Therefore we compare instead the absolute and the relative increases of the values and choose for this the two cultures with 5.0 mg N/l after four

Table 15. Experiment 929. Extinction ($e \times 10^3$), chlorophyll content ($\mu\text{g/ml}$), and cell concentration (cells/mm³). Determinations at the start of the experiment (0 days) and after 2 and 4 days.

929	mg N/l	Extinction			Chlorophyll			Cell concentration	
		0	2	4	0	2	4	0 days	4 days
A: 1	0.00	139	207	263	3.2	3.2	2.0	1,210 $\pm$ 128	1,450 $\pm$ 71
A: 3	0.10	139	204	—	3.2	2.8	—		
A: 7	0.25	139	213	272	3.2	3.1	2.6		
A: 8	0.50	139	222	292	3.2	3.5	2.8	1,210 $\pm$ 128	1,530 $\pm$ 109
A: 9	5.00	139	273	419	3.2	9.4	8.7	1,210 $\pm$ 128	3,200 $\pm$ 252
B: 1	0.00	83	99	134	0.7	0.2	0.0	530 $\pm$ 43	710 $\pm$ 99
B: 3	0.10	83	101	—	0.7	0.8	—		
B: 7	0.25	83	127	151	0.7	0.5	0.1		
B: 8	0.50	83	145	171	0.7	0.9	0.3	530 $\pm$ 43	960 $\pm$ 86
B: 9	5.00	83	225	342	0.7	6.3	6.2	530 $\pm$ 43	1,930 $\pm$ 117

days of the experiment (Table 16). The absolute increase proves to be about the same in both the parallel cases, possibly A: 9 has a certain predominance for extinction and cell concentration. B: 9, on the other hand, is decidedly superior regarding the relative increase, which is calculated by dividing the absolute increase by the corresponding initial value. This implies that both the growth and the formation of chlorophyll per inoculated cell were very much more active in B: 9 than in A: 9.

Table 16. Experiment 929. The absolute and the relative increase of extinction, chlorophyll content, and cell concentration in 929 A: 9 and 929 B: 9 after four days. Units as in Table 15.

	Absolute increase		Relative increase	
	A: 9	B: 9	A: 9	B: 9
Extinction	280	259	2.0	3.1
Chlorophyll	5.5	5.5	1.7	7.9
Cell concentration.	1,990	1,400	1.6	2.6

This difference in the relative increase could be imagined to depend on physiological differences between the two stock cultures used (927 A and 896 A). It is conspicuous, however, that the quotients for the corresponding

relative increases of the parallel cultures completely or nearly completely agree with the reciprocal quotients for their initial values (Table 17). If this agreement is not a coincidence it means that the amount of nitrate per cell is of great importance for the effect of a certain nitrate concentration on the growth and the formation of chlorophyll. At the start, 929 A: 9 had 6.0×10^7 cells and B: 9 had 2.7×10^7 cells and both cultures contained 250 μg N. Consequently each cell had at its disposal in A: 9 about 4×10^{-6} and in B: 9 about 9×10^{-6} μg of nitrate-nitrogen, i. e., in both cases less than the amount of nitrogen which FRANZEW found in the cell content of nitrogen-starved *Scenedesmus quadricauda* (see above, p. 93). It would not be surprising if the inoculated algae were capable of very quickly absorbing nitrate amounts of this order of magnitude.

Table 17. Experiment 929. The ratios of the initial values and those of the relative increases of extinction, chlorophyll, and cell concentration in 929 A: 9 and 929 B: 9 after four days.

	Initial values	Relative increases
	A: 9 B: 9	B: 9 A: 9
Extinction	1.7	1.6
Chlorophyll	4.6	4.6
Cell concentration.	2.3	1.6

In order to investigate more closely the effect of the initial cell concentration on the relative growth and formation of chlorophyll, I started, in immediate connection with the experiment just reported, and under the same conditions as this, two new experimental series (932 A and B). As inoculating material, the starvation-culture 896 B was used (parallel to the stock culture 896 A used in experiment 929), whereby inocula of different sizes were added to flasks containing 5.0 and 0.5 mg $\text{N}(\text{NO}_3)/\text{l}$ respectively. The procedure and the primary results are given in Table 18, while the values for the absolute and relative increases of extinction, chlorophyll content, and cell concentration after 3, 5, 7, and 9 days follow from Table 19.

Here, as in the previous experiment (No. 929), we find that 5.0 mg N/l has considerably greater effect than 0.5 mg/l on both the growth and the formation of chlorophyll. But the nitrate concentration alone is not decisive for the result. This becomes particularly obvious if we consider the values for the relative increase. The relative effect of the nitrogen content is greater the less the inoculum. This holds, in a different degree, for the

Table 18. Experiment 932. Extinction ($e \times 10^3$), chlorophyll content ($\mu\text{g/ml}$), and cell concentration (cells/mm³) for 0.5 and 5.0 mg N (NO₃)/l with different inocula.

Measurements at the start (0 days) and after 3, 5, 7, and 9 days.

932 A: 1—4. 50 ml of VIII (N = 500) per flask.

932 B: 1—4. 50 » » VIII (N = 5,000) » » .

Inoculations:

A: 1 and B: 1. 1.0 ml algal suspension from 896 B + 0.0 ml dist. water,

A: 2 » B: 2. 0.5 » » » » 896 B + 0.5 » » » ,

A: 3 » B: 3. 0.1 » » » » 896 B + 0.9 » » » ,

A: 4 » B: 4. 0.05 » » » » 896 B + 0.95 » » » .

932	Extinction					Chlorophyll				
	0	3	5	7	9	0	3	5	7	9
A: 1	176	252	301	330	370	3.4	3.3	3.1	1.9	1.1
A: 2	108	172	207	230	232	1.3	1.4	1.0	0.6	0.5
A: 3	60	70	103	112	112	0.3	0.5	0.4	0.4	0.4
A: 4	40	42	76	78	90	0.1	0.3	0.2	0.3	0.4
B: 1	176	380	552	654	730	3.1	7.8	7.2	6.4	4.7
B: 2	106	277	430	535	617	1.2	6.8	6.8	5.7	4.2
B: 3	60	90	301	421	491	0.3	2.7	5.6	5.0	4.5
B: 4	40	35	90	347	458	0.1	0.5	2.9	4.7	5.3

932	Cell concentration				
	0	3	5	7	9
A: 1	1,540 ± 140	1,940 ± 171	1,790 ± 204	2,340 ± 222	2,000 ± 160
A: 2	820 ± 139	940 ± 70	1,270 ± 171	1,550 ± 120	1,850 ± 129
A: 3	(160)				665 ± 96
A: 4	(80)				327 ± 20
B: 1	1,600 ± 181	2,660 ± 205	3,760 ± 200	3,980 ± 231	4,690 ± 268
B: 2	790 ± 88	2,430 ± 166	2,170 ± 310	2,220 ± 180	3,490 ± 201
B: 3	(160)	640 ± 59		2,560 ± 154	2,750 ± 108
B: 4	(80)			2,700 ± 204	2,690 ± 238

substance production (the extinction), the formation of chlorophyll, and the cell increase. The first mentioned is the least affected, and the most sensitive is the formation of chlorophyll. For 0.5 mg N/l we can simply establish an unbroken diminution of the chlorophyll content for the two largest inocula (negative values in Table 19). For 5.0 mg N/l the formation of chlorophyll was finished within only three days in B: 1 but perhaps

Table 19. Experiment 932. The absolute and the relative increase of extinction, chlorophyll content, and cell concentration after 3, 5, 7, and 9 days. Units as in Table 18.

932	Absolute increase				Relative increase			
	3	5	7	9	3	5	7	9
E x t i n c t i o n								
A: 1	76	125	154	196	0.43	0.71	0.88	1.11
A: 2	64	99	122	124	0.59	0.92	1.13	1.15
A: 3	10	43	62	62	0.17	0.72	1.03	1.03
A: 4	2	36	38	50	0.05	0.90	0.95	1.25
B: 1	204	376	478	554	1.16	2.14	2.71	3.14
B: 2	171	324	429	511	1.61	3.06	4.05	4.82
B: 3	30	241	361	431	0.50	4.01	6.02	7.19
B: 4	—5	50	307	418	—0.13	1.25	7.68	10.47
C h l o r o p h y l l								
A: 1	—0.1	—0.3	—1.5	—2.3	—0.03	—0.09	—0.44	—0.68
A: 2	0.1	—0.3	—0.7	—0.8	0.08	—0.23	—0.54	—0.61
A: 3	0.2	0.1	0.1	0.1	0.67	0.33	0.33	0.33
A: 4	0.2	0.1	0.2	0.3	2.00	1.00	2.00	3.00
B: 1	4.7	4.1	3.3	1.6	1.52	1.32	1.07	0.52
B: 2	5.6	5.6	4.5	3.0	4.66	4.66	3.75	2.50
B: 3	2.4	5.3	4.7	4.2	8.00	17.67	15.67	14.00
B: 4	0.4	2.8	4.6	5.2	4.00	28.00	46.00	52.00
C e l l c o n c e n t r a t i o n								
A: 1	400	250	800	460	0.26	0.16	0.52	0.30
A: 2	120	450	730	1,003	0.15	0.55	0.89	1.23
A: 3				505				3.15
A: 4				247				3.09
B: 1	1,060	2,160	2,380	3,090	0.66	1.35	1.49	1.93
B: 2	1,640	1,380	1,430	2,700	2.08	1.75	1.81	3.42
B: 3	480		2,400	2,590	3.00		15.00	16.19
B: 4			2,620	2,610			32.75	32.62

not even within nine days in B: 4. In all four cultures with this nitrate concentration, the absolute increase of the chlorophyll content was of the same order of magnitude (between 4.7 and 5.6 $\mu\text{g}/\text{ml}$), but the relative increase varied between 1.5 and 52.0. In an analogous way the absolute increase of the cell number in these four cultures (B: 1—B: 4) was fairly equal (between 2,590 and 3,090 cells/ mm^3), but the relative cell increase

was very different: between 1.9 cells per inoculated cell in B: 1 and about 33 in B: 4.

The ratios of the relative increases in B: 2—B: 4 and the same increase in B: 1 are compared in Table 20 with the reciprocal ratios for the initial values. The agreement — here, as in Table 17 — is as good as can be expected with respect to the large sources of error. It seems, therefore, incontrovertible that the relative increase is a direct function of the size of the inoculum. Strictly speaking it would be more surprising if this should not be the case. The absorption of suboptimal ions by the algae must form a decisive factor for the effect of these ions on the metabolism of the individual cells. The cell can only make use of the ions which it has absorbed, and the harder the competition for the available ions the less becomes the share of each cell. The experimental proof of this obvious fact is also of interest from an ecological point of view (see below, p. 101).

Table 20. Experiment 932. The ratios of the initial values and those of the relative increases of extinction, chlorophyll, and cell concentration in 932 B: 1—4. (For chlorophyll the maximal values have been used.)

	Initial values			Relative increases		
	B: 1	B: 1	B: 1	B: 2	B: 3	B: 4
	B: 2	B: 3	B: 4	B: 1	B: 1	B: 1
Extinction	1.7	2.9	4.4	1.5	2.3	3.3
Chlorophyll	2.6	10.3	31.0	3.1	11.6	34.2
Cell concentration.....	2.0	10.0	20.0	1.8	8.4	16.9

PRATT (1940) has studied the influence of the inoculum on the growth of *Chlorella vulgaris* in a culture medium which contained all the nutrients in adequate concentrations. He found that “the maximum population attained was independent of the size of the inoculum, but the rate of multiplication throughout the growth period . . . was greater for cultures initially containing a small number of cells than for those started with larger numbers” (p. 56). This is the same result as was here obtained in the experiments with *Scenedesmus quadricauda*. According to PRATT’s conception, however, the influence of the inoculum was dependent on “a growth-inhibiting substance, the amount of which varied directly with the number of cells,” being “carried into the cultures with the original inoculum” (p. 55). PRATT has later (see for instance 1942, 1944) given incontestable proof for such an inhibiting substance being formed in pure cultures of *Chlorella* and being there of great importance for growth. In his first paper (quoted

above) in the series "Studies on *Chlorella vulgaris*" the conclusions are not completely convincing. In the experiments carried out by the author, where the growth was limited by the supply of nitrogen, at least it appeared most probable that in the first place it was the competition of the cells for the nitrate ions which was decisive for the relative increase of both the growth and the chlorophyll formation.

In order to establish whether organic substances of the nature of enzymes or vitamins affected the utilization of the nitrate for the growth and chlorophyll formation of *Scenedesmus quadricauda*, experiment 952 was started under the same conditions as above. 12 flasks containing 50 ml of culture solution were inoculated with nitrogen-starved *Scenedesmus* from a parallel culture (896 A: 1) to the filter tube cultures used in the previous experiments. The flasks were supplied with 0.0, 0.5, 1.0, and 5.0 mg N/l, three flasks for each concentration, by means of different additions of $\text{Ca}(\text{NO}_3)_2$. To 952 A: 1—4 was then added 1.0 ml of distilled water, to 952 B: 1—4 0.5 ml of extract of bottom sediment from Erken + 0.5 ml of distilled water, and to 952 C: 1—4 was finally added 1.0 ml of soil extract. The results after 7 days are given in Table 21.

We can state very briefly that the additions of extract had no or very insignificant effect. The substance production was not affected at all, but

Table 21. Experiment 952. Nitrate additions without (952 A) and with simultaneous addition of extract of lake sediment (952 B) and of soil (952 C). Initial values: extinction ($e \times 10^3$)=50, chlorophyll content ($\mu\text{g/ml}$)=0.3, cell concentration (cells/mm³)=100 $\pm$ 40. The values in the table were obtained after 7 days.

952	mg N(NO_3)/l	Extinction	Chlorophyll	Cell concentration
A: 1	0.0	75	0.3	125 $\pm$ 62
A: 2	0.5	136	0.1	
A: 3	1.0	171	0.6	750 $\pm$ 121
A: 4	5.0	363	5.5	1,840 $\pm$ 155
B: 1	0.0	93	0.2	300 $\pm$ 30
B: 2	0.5	150	0.3	
B: 3	1.0	168	1.2	820 $\pm$ 98
B: 4	5.0	363	6.1	2,010 $\pm$ 127
C: 1	0.0	108	0.3	465 $\pm$ 80
C: 2	0.5	155	0.3	
C: 3	1.0	181	0.6	1,120 $\pm$ 96
C: 4	5.0	376	5.9	2,560 $\pm$ 157

the cell division and chlorophyll formation showed somewhat higher values in the presence of extract. These small differences in relation to the control cultures could, however, probably be attributed to the extracts' own content of nitrate or other assimilable nitrogen source. Consequently there is no reason to assume that the nitrate dependence of *Scenedesmus* in nature is affected by thermostable organic substances occurring there.

Further information on the nitrate dependence of chlorophyll formation will be given in the next section (p. 108 and following).

Discussion.

We first consider the experimental result that the amount of nitrate per cell plays an essential role in the effect of a certain nitrate concentration on the cell increase and substance production. This fairly obvious fact must be assumed to exist for the other culture factors also (cf. phosphate experiment 1020, p. 71), and ever greater importance must be attached to it, the less the ionic concentration and the greater the population density.

The competition of the algae for the available nutrients is of course much more intensive in an eutrophic lake than in an oligotrophic. In return, the concentrations of the nutritive substances are most often higher in a lake of eutrophic type. But during periods of high phytoplankton development the supply of, e. g., phosphate and nitrate not seldom becomes very reduced at the same time as the density of plankton algae increases. One can ask with reason whether the individual plankton algae under such circumstances have better nutritive conditions in a "nutrient-rich" lake than in a "nutrient-poor." The former has, of course, a higher standing crop and can, therefore, even for low concentrations of nutrients (e. g., at the end of a high production) produce a larger absolute increase of cell number and plankton substance per unit volume than can the oligotrophic lake with the same nutrient supply during the same period. But by that it is not said that the relative growth, consequently the increase per cell, is always greater in highly productive waters than in those poor in specimens. The results of the previous experiments (Nos 929 and 932) give ground for the supposition that this should not always be the case.

In the previous section we established (p. 74) that *Scenedesmus quadricauda*, despite complete absence of phosphate in the solution, could continue the cell increase and substance production for a very long time. We have now seen that during a shorter time this is possible even with a deficiency of nitrate. In the following sections we shall find (pp. 105, 119) that neither does the absence of iron, magnesium, or potassium cause *S. quadri-*

cauda to cease its growth immediately. This ability, when necessary, to make use of the cells' own recourses, accumulated during periods of good nutrient supply, was observed by FRANZEW (1932) with both *S. quadricauda* and *Pandorina morum*, and by KETCHUM (1939) with *Chlorella pyrenoidosa* and the marine diatom *Nitzschia closterium*. It appears, therefore, probable that the ability for a certain "internal economy" can be a general property of the plankton algae, which thereby should have better possibilities even in nature to come through periods of low nutrient supply. The debts of the cells may be covered in the hypolimnion or on the bottoms of the lakes, where a perpetual liberation of nutritive ions takes place by the bacterial decomposition processes. At circulation periods such opportunities of restoring the cellular resources should be offered the plankton algae even in the surface layers. If these suggestions are correct, we have not to expect immediate responses of phytoplankton growth to the variations of the nutrient supply. RILEY (1940 p. 295) found that the best correlation between chlorophyll content and the variations of N and P was obtained for a lag period of about a week.

Contrary to the cell increase and the substance production, the formation of chlorophyll ceases very quickly for nitrate deficiency; the chlorophyll content of the cells then begins to decrease (p. 97, see also Table 14 and fig. 5). Even with 0.5 mg of nitrate-nitrogen per litre, i. e., a nitrate content too high to exist in the epilimnion of most lakes, the chlorophyll formation could be completely prevented by a high algal density (Table 19). This effect can be assumed to be a reality in eutrophic waters, where the populations certainly do not reach the algal concentrations of the cultures but where instead the nitrate content is usually lower than in the experiment. A proof of the correctness of this supposition I cannot submit, but by examinations and comparisons of fresh plankton samples from oligotrophic and eutrophic lakes I have often received the purely subjective impression, especially during the summer, that the Chlorococcales are paler (poorer in chlorophyll) in the latter mentioned lakes.

In the last ten years one has tried to obtain a measure of the organic matter formed by phytoplankton by determining the chlorophyll content of natural waters (see, e. g., KOZMINSKI 1938, DEEVEY 1940, HANDKE 1941, GESSNER 1944). The results so far obtained are very encouraging, but the theoretical foundation of the method is not yet completely elucidated. The experiments described above have shown that chlorophyll formation and substance production (and cell increase) do not at all follow parallel courses for a lack of nitrate. Under different conditions the same cell number of a certain species, e. g., *Scenedesmus quadricauda*, can contain different amounts of both chlorophyll and other organic substances.

Further, the relation between the amount of chlorophyll and the content of organic matter in the cells is certainly different for various species. These and other apprehensions suggest that the chlorophyll content of lake waters cannot be used as a reliable measure of the standing crop of plankton algae. On the other hand one can hope to get a measure of the photosynthetic activity of the total phytoplankton by chlorophyll determinations of lake waters. GESSNER (1943) found that the assimilation number, i. e., the ratio of photosynthesis to chlorophyll, in 10 out of 12 investigated lakes lay between 4 and 6. According to VAN HILLE (1938), however, the assimilation number of *Chlorella pyrenoidosa* was decreased by, among other things, nitrate deficiency. Further investigations are needed in order to solve whether this also holds for plankton algae under natural conditions.

VII. Iron as a Limiting Factor for Growth.

Introduction.

Next to phosphorus and nitrogen, iron is often considered to be one of the most important chemical factors for the development of phytoplankton (cf. NAUMANN 1932 pp. 10 and 14). The most energetic advocate of iron has been USPENSKI (1927) who put forward the theory that iron would be absolutely the dominating factor for the distribution of the algae. He based this opinion on both ecological observations and experimental results (see also USPENSKI & USPENSKAJA 1925) and particularly emphasized the importance of the poisonous effect of the iron, which was decreased by organic substances as, e. g., Na-citrate. PRINGSHEIM (1934) has subjected the premises of USPENSKI's theory to a detailed examination. He found them obscure, and repudiated the altogether too one-sided conception of USPENSKI. On the other hand, PRINGSHEIM accepted the idea of the detoxication (*Entgiftung*) of the iron by "buffering" with complex-forming organic compounds; according to my opinion, however, this phenomenon should be interpreted in another way (cf. above p. 42).

The role of iron as a limiting factor for the growth of plankton algae has not been the object of more detailed studies. Concerning the marine phytoplankton, GRAN considers that "the soluble iron compounds, washed out with 'humus'-compounds from the shores, may give the explanation of the relative productivity of coastal waters in comparison with the open ocean" (1933 p. 169). His culture experiments (1931, 1933) show that the addition of iron certainly increases the growth, but this increase seems to be of secondary importance in relation to the effects of nitrate and phosphate additions. HARVEY (1945 p. 136) also finds that "there are grounds for expecting that the growth of phytoplankton may be limited in some parts of the open oceans by an insufficient supply of iron." According to HARVEY (1937), marine diatoms can utilize colloidal and larger particles of ferric hydroxide and ferric phosphate for their growth, an ability which should be of great importance in the open oceans, where the iron possibly occurs mainly in these forms.

In fresh waters the quantity and the form of occurrence of iron are certainly considerably more diversified than in the oceans. However, as yet, there are very few definite particulars about this at our disposal (cf. above p. 29). Neither do we know much about the iron requirements of freshwater plankton algae and their ability to assimilate different forms of iron. In the next few pages I shall try to present a contribution to some of these important questions.

Influence of suboptimal iron concentrations on growth and chlorophyll formation of *Scenedesmus quadricauda*.

In the experiments preceding the composition of culture solution VIII we have already observed that the nature of the iron source is of great importance for the success of the cultures (see p. 45). Ferric citrate in combination with equal amounts by weight of citric acid proved to give satisfactory results, and in the following experiments the iron has been added in this manner. The iron stock solutions were always autoclaved separately and kept in the dark to avoid hydrolysis.

In order to obtain iron-starved material the same filter tube method was used as for the phosphate and nitrate experiments (cf. p. 74 and fig. 12); the stock cultures were swilled through with iron-free solution, VIII ($\text{Fe} = 0$), about three times per week. For these cultures, which received large inocula, it proved to need a very long time, about six months or even longer, before the growth ceased because of the absence of iron. According to the photometric determinations of the extinction, the amount of substance had then increased at least 20 times since the iron was removed from the solution. The formation of chlorophyll ceased earlier, but first after 3—4 months the cultures began to be chlorotic (greenish yellow).

Consequently, *Scenedesmus* in the absence of iron (as in the absence of phosphate and nitrate) can continue the increase by using the cell's own store. FRANZEW (1932) made the same observation and found that the iron content after three days' iron starvation decreased from $43 \times 10^{-6} \mu\text{g}$ to $5 \times 10^{-6} \mu\text{g}$ Fe/cell (loc. cit., Tables 3 and 4), while the cell number increased nearly three times and the dry weight of the algal material increased from 12.8 to 31.3 mg. Obviously under favourable conditions the cells can store up a considerable surplus of iron above the actual need.

I have carried out several series of experiments in culture solution VIII ($\text{Fe} = 0$), inoculated from iron-starved stock cultures. The iron concentration was varied by graded additions of solutions with ferric citrate—citric acid in suitable concentrations. Distilled water was also added to

each culture, which consisted of 50 ml of culture solution in a 100 ml flask, in the requisite amounts to give the same volume to all the parallels in the series. The temperature was 25° C, and the time of illumination was 20 hours daily. 10 ml of algal suspension were taken out for each measurement. The sample was first photometrically measured, after which the cell number was determined by counting a few drops in a BÜRKER chamber. The algae were then separated by centrifuging and extracted with boiling methanol for determination of chlorophyll.

Experiment 889 B can be quoted first. The results are illustrated in figures 18, 19, and 20. The curves for 0 $\mu\text{g Fe/l}$ show that the substance production (according to *Z*) and the cell number continued to increase

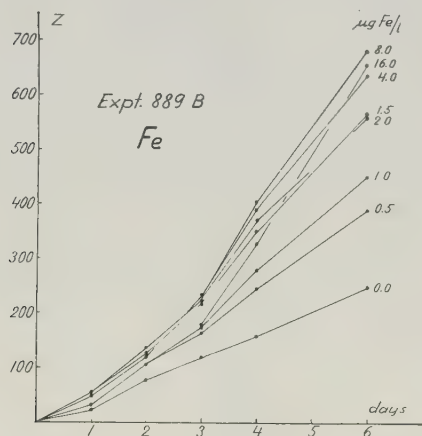


Fig. 18.

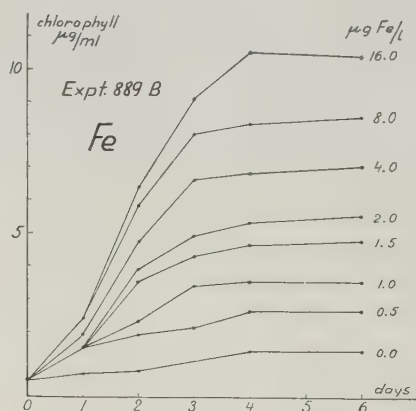


Fig. 19.

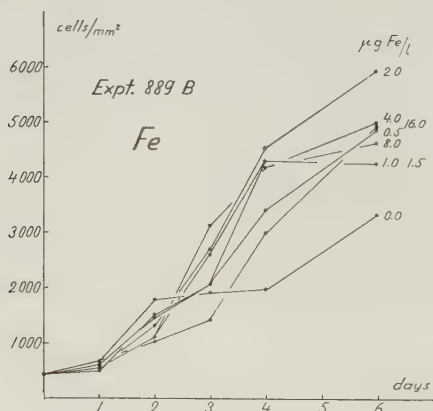


Fig. 20.

Growth (extinction values, *Z*, fig. 18; and cell numbers, fig. 20) and chlorophyll formation (fig. 19) of iron-starved *Scenedesmus quadricauda* for different concentrations of iron.

despite several months' iron starvation, while no noteworthy formation of chlorophyll took place without addition of iron. The latter is consequently more dependent on the supply of iron than the growth is.

All three methods of measurement registered a distinct positive effect even for 0.5 $\mu\text{g Fe/l}$. The immediate utilization of such a low iron concentration for increased growth and formation of chlorophyll shows that the iron debt of the cells is different in character from the phosphate debt, where sufficient restoration to give growth demanded a fairly long time and high concentrations (p. 107).

For increasing iron concentrations growth and chlorophyll formation increased in somewhat different ways. For the substance production and particularly for the cell increase, the courses of the curves are partly rather erratic. 2 and 4 $\mu\text{g Fe/l}$, e. g., gave higher cell yields than 8 and 16 $\mu\text{g Fe/l}$. This must be interpreted as an expression of the connection between iron concentration and growth being of a complicated character. On the other hand the chlorophyll formation, the curves of which are very regular, seems to be a more direct function of the iron supply.

But in experiment 889 B the iron was not the only factor affecting the chlorophyll formation. This appears from fig. 19 but is yet clearer if we calculate the chlorophyll yield per $\mu\text{g Fe/l}$ (Table 22). We then find that the formation of chlorophyll was approximately directly proportional to the iron concentration only for the lowest concentrations, up to 2 $\mu\text{g Fe/l}$. Above this a given increase of the iron content gave a less increase of the chlorophyll formation. This was obviously limited by one or more other factors than the supply of iron, if the growth attained high levels.

In the previous section we established that the supply of nitrogen is of great importance for the chlorophyll formation of *Scenedesmus quadri-*

Table 22. Experiment 889 B. The increase of the chlorophyll content after six days (the values obtained by subtraction of the chlorophyll content of the control culture) and the chlorophyll yield per $\mu\text{g Fe/l}$.

Iron concentration $\mu\text{g/l}$	Increase of chlorophyll $\mu\text{g/ml}$	Yield of chlorophyll per $\mu\text{g Fe/l}$
0.5	1.2	2.4
1.0	2.1	2.1
1.5	3.3	2.2
2.0	4.1	2.1
4.0	5.6	1.4
8.0	7.1	0.9
16.0	8.9	0.6

cauda. It is therefore plausible to suspect that the relatively low nitrate content (10.2 mg N/l) in culture solution VIII was the foremost cause for the decreasing yields of chlorophyll at higher growth. In order to study this question more closely, and at the same time to decide whether the phosphate content as well has an effect on the formation of chlorophyll, experiment 928 was started with additions of nitrate, phosphate, and iron according to the following combinations:

Expt. 928	N mg/l	P mg/l	Fe μg/l
1	0	0	0
2	10	0	0
3	10	1	0
4	10	1	2
5	10	1	4
6	10	0	4
7	5	1	4
8	1	1	4
9	0.5	1	4
10	0	1	4

For inoculation I used *Scenedesmus quadricauda* from a highly productive filter tube culture (911 B) which had been swilled through with VIII (Fe = 0) 2 or 3 times each week during eight months. 5 ml of algal suspension were repeatedly washed in a sterile filter tube with culture solution VIII (N, P, Fe = 0), i. e., a culture solution without phosphate, nitrate, and iron. The algae were then suspended in 500 ml of this solution to which ferric citrate—citric acid corresponding to a concentration of 4 μg Fe/l was added. The solution was divided into 50 ml in each of 10 flasks (928: 1—10) which contained $\text{Ca}(\text{NO}_3)_2$, K_2HPO_4 , and ferric citrate—citric acid, in different combinations according to the scheme above. The flasks were placed in a photothermostat at 25° C and with an illumination time of 20 hours per day. The extinction values, chlorophyll contents, and cell concentrations were determined at the beginning of the experiment and after 2, 3, and 4 days. The results are shown in Table 23.

According to the extinction values, the substance production showed no significant reaction whatever to the different combinations. The highest values were obtained — certainly completely accidentally — in the control culture (No. 1). The increase of extinction of the other cultures varied fairly arbitrarily from case to case.

The cell concentrations were not determined for all the cultures. However, it follows from the available values that the positive effect of the iron additions was eliminated by a diminution of the nitrate content which then became the decisive factor for the cell increase.

Table 23. Experiment 928. The effects of nitrate, phosphate, and iron in different combinations on substance production (extinction), chlorophyll formation, and cell increase of *Scenedesmus quadricauda*. Initial values: Extinction ($e \times 10^3$) 588, chlorophyll content ($\mu\text{g/ml}$) 8.4, cell concentration (cells/mm³) 6,050 $\pm$ 233. Determinations after 2, 3, and 4 days. Concerning the combinations of N, P, and Fe, see p. 108.

928	Extinction			Chlorophyll			Cell concentration	
	2	3	4	2	3	4	2	4
1	893	933	1,030	9.1	9.3	8.4	6,850±204	8,100±334
2	776	840	943	14.3	14.9	15.5		
3	805	897	1,009	13.7	14.4	15.2		
4	745	811	941	15.7	17.0	17.2		
5	818	921	1,024	16.9	18.0	18.7	8,940±441	10,800±533
6	773	844	946	17.0	17.9	18.7		
7	820	926	1,009	14.6	15.4	15.7	8,300±441	8,400±730
8	832	909	977	12.8	12.4	12.8		
9	856	926	994	10.3	11.5	11.9	7,040±177	7,380±350
10	838	902	1,000	11.5	11.7	11.5	6,560±266	7,100±342
928	Increase of chlorophyll			Colour of culture after four days			Increase of cell concentration	
	2	3	4				2	4
1	0.7	0.9	0.0	yellow			800	2,050
2	5.9	6.5	7.1	green				
3	5.3	6.0	6.8					
4	7.3	9.6	8.8	deep-green				
5	8.5	9.6	10.3					
6	8.6	9.5	10.3				2,890	4,750
7	6.2	7.0	7.3	yellowish-green			2,250	2,350
8	4.4	4.0	4.4	yellow				
9	1.9	3.1	3.5					
10	3.1	3.3	3.1				990	1,330
							510	1,050

The chlorophyll formation was lowest in the control culture where we notice an inconsiderable increase of the chlorophyll content during the first 2—3 days. This culture contained (like all the other flasks) from the beginning 4 μg Fe/l. According to the previous experiment (889 B, see Table 22) this iron concentration should be sufficient for a considerable chlorophyll increase.

That it is the lack of nitrogen which is the cause of the inconsiderable and passing formation of chlorophyll in No. 1 is proved by the course in No. 2, the culture solution of which only differed from the first mentioned

culture by obtaining 10 mg N/l at the start. For this the chlorophyll content could increase by $5.9 \mu\text{g/ml}$ in only two days and then it continued to increase gently to the end of the experiment. The increase during four days ($7.1 \mu\text{g}$ chlorophyll/ml) was almost the same as in experiment 889 B in the same time with the corresponding Fe-concentration (see fig. 19, curve for $4 \mu\text{g}$ Fe/l). The absence of phosphate consequently seems to have no immediate effect on the formation of chlorophyll. This follows also from a comparison between culture No. 2 (without phosphate) and culture No. 3 (with 1 mg P/l), the chlorophyll values of which are practically the same. The phosphate store of the cell itself was obviously sufficient to provide for the chlorophyll formation in the phosphate-free medium (cf. p. 74).

Cultures Nos 4 and 5 had 10 mg N/l and 1 mg P/l and differed in that No. 4 obtained an addition of $2 \mu\text{g}$ and No. 5 an addition of $4 \mu\text{g}$ Fe/l; thus they had at the start a total of 6 and 8 μg Fe/l respectively. The effect of the iron on the chlorophyll formation is quite obvious.

Culture No. 6 was identical with No. 5 except that it lacked phosphate. This lack, in agreement with what recently appeared concerning Nos 2 and 3, had no importance for the formation of chlorophyll. The chlorophyll contents of both the cultures were in agreement for all three determinations; they were the highest in the whole series, which also appeared directly from the dark green colours of both these cultures.

In the cultures Nos 7—10 the phosphate and the iron were constant (1 mg P/l and $4+4 \mu\text{g}$ Fe/l), while the nitrate was graded from 5 to 0 mg N/l. If we add No. 5, which has 10 mg N/l with the same concentrations of P and Fe as in the cultures 7—10, the nitrate additions include the interval 0—10 mg N/l. We notice that the chlorophyll increase in the nitrate-free culture No. 10 rose to (at least) $3.1 \mu\text{g/ml}$. This increase must be based on the nitrogen store of the cells themselves, because in the experiments with nitrate-starved inocula (experiment 929, Table 15, and experiment 952, Table 21) no chlorophyll formation whatever was obtained without nitrate addition. If we subtract 3.1 from the values for the increase of the chlorophyll content after four days in the cultures 928: 5, 7, 8, and 9, we should obtain more adequate expressions for that chlorophyll increase which was dependent on the nitrate concentration of the solution:

Expt. 928	mg N/l	μg chlorophyll/ml
9	0.5	0.4
8	1	1.3
7	5	4.2
5	10	7.2

For the three lowest concentrations the proportionality between the nitrate content and the nitrate-dependent chlorophyll increase is as good as can be expected with regard to the experimental errors. Such a proportionality was also established by VAN HILLE (1938 p. 730). In culture 928:5 on the other hand the effect of the nitrate on the formation of chlorophyll was considerably less than that predicted from the proportionality. But in this culture the iron concentration also became a limiting factor for the chlorophyll formation.

When the nitrate supply is the only limiting factor we can obviously depend on a maximal chlorophyll formation of approximately 1 μg chlorophyll per ml for each mg nitrate-nitrogen per litre, i. e., 1 mg chlorophyll per mg of nitrate-nitrogen. (We disregard in this the chlorophyll formation which can take place owing to the nitrogen store of the cells themselves.) This agrees well with the results of the experiments 929, 932, and 952 described in the previous chapter (see Tables 15, 19, and 21) and it gives a good explanation of the cessation of the chlorophyll formation for 10.5 μg chlorophyll/l in experiment 889 B (fig. 19). In the epilimnion of the lakes, where the nitrogen supply is not the only minimum factor, the ratio of chlorophyll to nitrogen always seems to be less than 1 (see the chlorophyll determinations of DEEVEY 1940, GESSNER 1943, 1944, HANDKE 1941, KOZMINSKI 1938, RILEY 1940).

On the basis of the results of experiment 928 we can consequently establish that the lack of nitrate was the foremost and perhaps the only cause for the proportionality between chlorophyll yield and iron content in experiment 889 B ceasing at the higher iron concentrations, i. e., higher growths (see Table 22). The experiment was therefore repeated with a modification of culture solution VIII (called VIII'), where the content of nitrate was increased from 10.2 to 50.2 mg N/l by addition of NaNO_3 . In experiment 980 A the iron was graded up to 100 μg Fe/l. The stock culture, the inoculum, and the experimental conditions were otherwise the same as in experiment 889 B. Of the results, only the chlorophyll formation will be quoted here (fig. 21). The substance production and the cell increase behaved in an analogous way to experiment 889 B.

The curves for chlorophyll formation in experiment 980 A to a large extent follow the same regular course as in experiment 889 B. But the direct proportionality between iron concentration and chlorophyll increase extends here over a considerably greater range of concentration, namely between 4 and 10 μg Fe/l (Table 24). The proportionality would probably include even 20 μg Fe/l if the experiment had been continued until the chlorophyll formation ceased for this concentration also. With an extension of the experimental time the chlorophyll yields of both of the highest

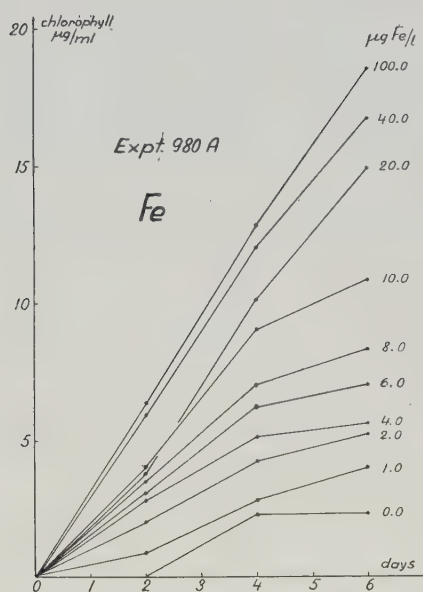


Fig. 21.

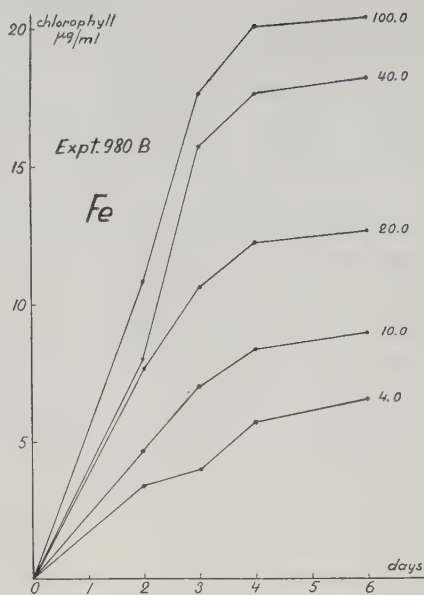


Fig. 22.

Chlorophyll formation of iron-starved *Scenedesmus quadricauda* for different iron concentrations in nitrate-enriched medium without (fig. 21) and with (fig. 22) addition of bicarbonate.

concentrations would also have been higher, but scarcely high enough to maintain the same proportion to iron as the yields of the lower concentrations, since the nitrate supply began to interfere as limiting factor.

The interference of factors limiting growth or assimilation has been studied in many different connections with various objects. LUNDEGÅRDH (1931 p. 204) has given the following definition of "the law of relative effects": "The more nearly a factor is in minimum in relation to other factors acting upon the plant, the greater is the relative influence of a change of that factor upon the growth of the plant. As a factor increases in intensity, its relative effect upon the plant decreases; and when the factor is in the region of its maximum the effect of a change upon the plant is nil." In complete agreement with this law, both of the lowest iron concentrations in expt. 980 A had a greater relative effect on the chlorophyll formation than the nearest higher concentrations (Table 24). The same holds for the lowest concentration in expt. 889 B (Table 22).

Both the rate and the final yield of the chlorophyll formation were higher in expt. 889 B than in expt. 980 A. In these two cases the culture solutions were certainly not exactly the same, but even in series which were carried out at different times under, as far as could be judged, identical conditions and inoculated from the same stock culture it has often been

Table 24. Experiment 980 A. The increase of the chlorophyll content after six days (the values obtained by subtraction of the chlorophyll content of the control culture) and the chlorophyll yield per $\mu\text{g Fe/l}$.

Iron concentration $\mu\text{g/l}$	Increase of chlorophyll $\mu\text{g/ml}$	Yield of chlorophyll per $\mu\text{g Fe/l}$
1	1.7	1.7
2	2.9	1.5
4	3.3	0.8
6	4.7	0.8
8	6.0	0.8
10	8.5	0.8
20	12.6	0.6
40	14.4	0.4
100	16.2	0.2

difficult to obtain numerically reproducible results. Expt. 980 A was carried out three months after expt. 889 B, and it can therefore appear probable that the differences depended on the different ages of the inocula. I will, however, point out that the stock culture was cultivated in filter tubes with repeated changing of the culture solution. The differences must be considered to be due to physiological changes within the cells, which we do not yet know and therefore cannot yet control. This lack of physiological constancy has also presented difficulties to me on other occasions in comparisons of results from non-simultaneous experimental series, even if these were inoculated with similarly treated and equally old material from one and the same clone culture. With identical simultaneous parallel experiments, on the other hand, I have met such discrepancies less often.

We have established earlier (p. 55) that additions of bicarbonate gave rise to a considerable increase of the rate of growth. In order to decide if the same holds for the formation of chlorophyll, I carried out in expt. 980 B parallels to some of the higher iron concentrations in expt. 980 A. To each of these parallel cultures, containing 50 ml of algal suspension, there was added at the start 250 mg NaHCO_3 , after which the flasks were closed with rubber stoppers. The results of the chlorophyll determinations are shown in fig. 22.

The addition of the bicarbonate resulted in a very great increase of the rate of chlorophyll formation. The final level of the content of chlorophyll was reached in only four days, while in expt. 980 A for the higher iron concentrations this was not the case even two days later. A calculation of

the relative effect of the iron on the formation of chlorophyll cannot be made here in the same way as in Tables 22 and 24, while I neglected to arrange a control culture without iron additions in this experiment.

Biological determination of the concentration of assimilable iron in lake water.

As stated above (p. 30), only a small fraction of the total iron content of lake waters exists in reactive form. I shall give an account here of some of my preliminary experiments to determine the amount of assimilable iron in lake waters by means of iron-starved cultures of *Scenedesmus quadricauda*.

For the biological iron determination three 50 ml samples of lake water received K_2HPO_4 and $Ca(NO_3)_2$ according to the concentrations in culture solution VIII (0.89 mg P/l and 10.2 mg N/l respectively). The flasks were then inoculated with *Scenedesmus quadricauda* from an iron-starved filter tube culture. At the same time, and with the same inoculum, a series was started with graded iron additions in culture solution VIII ($Fe = 0$). The experiments were carried out in a photothermostat at $25^\circ C$ and with illumination for 20 hours per day.

The tested water was taken from the surface of the lake Skärsjön on November 21st, 1945. The average value of my earlier determinations (with the thiocyanate method) of total iron content in the surface water of Skärsjön is 0.12 mg Fe/l. The water sample mentioned, according to phenanthroline analysis on the residue after ignition, contained 0.10 mg total iron per litre; 0.01 mg Fe/l reacted with phenanthroline on direct analysis also. The sample was stored in a refrigerator ($+4^\circ C$, dark) without addition of chemical preservatives.

The water was tested with *Scenedesmus* on two different occasions. The first experiment (835 B) was carried out from December 3rd to 10th, 1945, at the same time as the iron-graded series No. 829; the second experiment (912 C) was carried out from March 7th to 11th, 1946, in parallel with series 910 G. In the first case the extinction of the cultures was determined, in the latter their chlorophyll content. In figures 23 and 24 the results (average values) are given for the testing experiments and for those concentrations of the parallel series which are of interest in this connection.

The substance production after four days was somewhat higher in the lake water than in the parallel culture containing $8 \mu g$ Fe/l. In the later experiment, the chlorophyll formation during the first four days was somewhat larger in the tested sample than in the parallel culture with $8 \mu g$ Fe/l; then

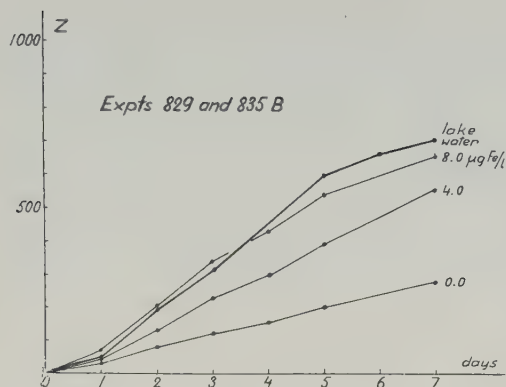


Fig. 23.

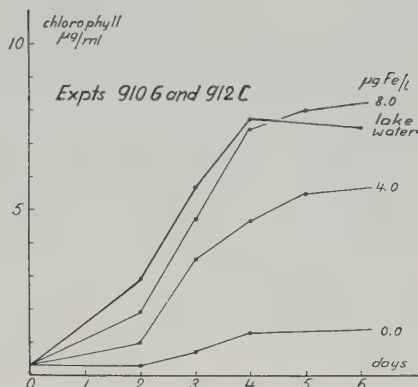


Fig. 24.

Growth (extinction values, Z, fig. 23) and chlorophyll formation (fig. 24) of iron-starved *Scenedesmus quadricauda* in lake water (enriched with N and P) and synthetic medium (with different iron concentrations).

followed, during the next two days, a diminution of the chlorophyll content in the lake water culture, whereby at the end of the experiment this lay somewhat below that of the parallel culture mentioned. With the guidance of our previous result concerning the importance of the nitrate content for chlorophyll formation, we can assume that this diminution depended on the lack of nitrate. A higher nitrate concentration (e. g., 50 mg N/l) than that used here (10.2 mg N/l) is therefore to be recommended for continued experiments of this nature.

Consequently it follows, from both the increase of extinction and the formation of chlorophyll, that *Scenedesmus quadricauda* could make use of somewhat more than 8 $\mu\text{g Fe/l}$ in the surface water of Skärsjön. This value is practically identical with the content of reactive iron (10 $\mu\text{g Fe/l}$) according to the direct phenanthroline determination. The agreement must be pointed out as very good, when one is reminded of the unavoidable and certainly fairly large experimental errors. Thus the lake water had to be concentrated ten times on a water bath in order that a direct analysis with phenanthroline might be possible. With this, of course, alterations of the reactive iron content could occur. Furthermore the content of reactive iron, even after the concentration of the water, lay at the lower limit of the range of measurement for phenanthroline (see fig. 6).

The biological method used here is obviously at least ten times more sensible than the phenanthroline analysis, but it is perhaps ten times more troublesome. It requires, of course, considerably longer time than a chemical analysis, even if the time of the experiment can certainly be decreased by suitable addition of bicarbonate (p. 113). The greatest difficulty,

however, lies in the fact that even one and the same starvation culture does not always give numerically reproducible results on different occasions (p. 113), even if this seems to have been the case in the two experiments quoted here. It is therefore scarcely probable that one can draw a usable calibration curve for the biological iron analysis. Instead one must evade this difficulty by arranging, each time, in parallel with the testing experiment itself, a series with stepped iron concentrations in a synthetic solution. This is a long-winded procedure but on the other hand no efforts seem to be too great when they are to widen our inconsiderable knowledge of the content of assimilable iron of natural waters.

Discussion.

According to experiment 889 B (figs 18—20) one would be inclined to regard 16 $\mu\text{g Fe/l}$ or a little higher iron concentration as optimal for the growth and chlorophyll formation of *Scenedesmus quadricauda*. According to experiment 980 A (fig. 21), on the other hand, the optimum of the iron seems to lie at about 100 $\mu\text{g Fe/l}$ or somewhat higher. The only difference between the two experiments was that the nitrate content in the first series was 10 $\mu\text{g N/l}$ and in the latter 50 $\mu\text{g N/l}$.

This is an example of the actual optimum interval of a limiting factor not having a fixed position. Its level must certainly be primarily dependent on the individual requirement of the organism in question, but it is also affected to a high degree by the environmental conditions. For each limiting factor one can distinguish between "potential (absolute) optimum" and "actual (relative) optimum." The former can only be realized when all the environmental factors lie within such favourable ranges of concentration that none of them limits the rate of growth. This should then be completely determined by the rate of the endogenous processes. Under such extremely favourable conditions, which may nowhere near exist in nature and perhaps cannot at all be realized more than asymptotically, the actual optimum of each factor is identical with its potential optimum. The position of this is fixed (absolute) for each organism. On the other hand the actual optimum is also dependent on the other environmental factors; it is therefore movable (relative). Its level becomes lower the more one, or some, of the other factors are limiting.

All this, from a physiological point of view, is so obvious that the quoted reflections can appear superfluous. They have only given a restatement of the law of relative effects of the limiting factors (p. 112). However, one often meets in ecological papers the idea that each organism should have a

constant optimum value for each factor (cf., e. g., GROTE 1934 pp. 8—11, THIENEMANN 1941 p. 101). This is correct concerning its absolute optimum, but does not hold for the actual optimum. Particularly under natural conditions it is, of course, the level of the latter which is of greatest interest in the estimation of the present conditions of growth. Unfortunately one most often meets great difficulties or it is impossible to establish the optimum curve of a factor under just those conditions which otherwise occur in nature. We can, however, by deduction from the experimentally found results, draw conclusions concerning the connection between environment and organism under natural conditions.

For *Scenedesmus quadricauda* the lower limit of the absolute optimum of the iron probably lies higher than $100\text{ }\mu\text{g Fe/l}$. In the epilimnion of most lakes the content of assimilable iron may be considerably lower than $100\text{ }\mu\text{g/l}$. Despite this, according to my opinion, the iron does not as a rule act as a limiting factor for the growth of *Scenedesmus quadricauda* under natural conditions; in any case not in all those waters, i. e. the great majority of lakes, where the content of assimilable phosphorus does not rise higher than about $20\text{ }\mu\text{g P/l}$ and the content of assimilable nitrogen does not exceed about 0.5 mg N/l . Then the growth, on account of the lack of phosphorus and nitrogen, cannot even reach the extent which in my experiments was obtained for only $0.5\text{--}1.0\text{ }\mu\text{g Fe/l}$, when the iron was the only limiting factor. Consequently it is very probable that the content of assimilable iron, though as a rule very low, usually lies in nature above the lower limits of the actual iron optima of *Scenedesmus quadricauda*.

The case is otherwise in those waters which, owing to the nutrient enrichment by human habitation, can give fairly high productions of *S. quadricauda* and other Chlorococcales. The concentration of assimilable iron in such waters should be particularly low (in the first instance due to their high pH). Moreover the level for the actual iron optimum must be raised by the better supply of phosphorus and nitrogen. It is therefore probable that in such cases the iron can become a limiting factor of importance for the development of plankton algae. These can perhaps, to a certain extent, then counteract the lack of iron and the strong competition for it by using their cellular iron store for the growth and the formation of chlorophyll.

VIII. Influence of Magnesium and Potassium Deficiency on the Growth of *Scenedesmus quadricauda*.

Magnesium and potassium belong to the elements necessary for life, but they are not considered to be limiting factors for the development of the plankton algae in lakes. So far I have only studied their effect on the growth of *Scenedesmus quadricauda* in rather few experiments, but a part of the results thereby obtained is not without interest.

Here, as in the experiments described above, I have used starvation cultures for inoculation. In these the lack of magnesium and the lack of potassium manifested itself in different ways. If the complete culture solution VIII in a filter tube culture with highly productive *Scenedesmus* is sucked away and then replaced with magnesium-free culture solution of otherwise practically the same composition — VIII ($\text{Mg} = 0$), the sulphate added as Na_2SO_4 — the increase of the extinction values diminishes very soon, ceasing completely within only two weeks. At the same time the culture becomes pale, and finally colourless.

On the other hand, in a potassium-starved *Scenedesmus* culture — in culture solution VIII ($\text{K} = 0$), the phosphate added as Na_2HPO_4 — the substance production continues several months after the removal of the potassium from the solution. The green colour also is maintained considerably longer than in a magnesium-starved culture and first becomes pale when the substance production and the cell increase begin to cease.

Consequently, with regard to the visible effects, magnesium starvation is reminiscent of nitrate starvation, while potassium starvation resembles phosphate starvation. That lack of magnesium should quickly affect the chlorophyll formation is easy to understand, since magnesium is the central atom in the chlorophyll molecule. On the other hand it does not seem to be known more exactly in which manner nitrate, phosphate, potassium, and iron contribute to the various processes involved in the chlorophyll synthesis (cf. RABINOWITCH 1945 pp. 336, 428).

The connection between the magnesium concentration and the growth of *Scenedesmus quadricauda* was studied in experiment 668. To each of 14 culture tubes were added 9.5 ml of culture solution VIII ($\text{Mg} = 0$) and —

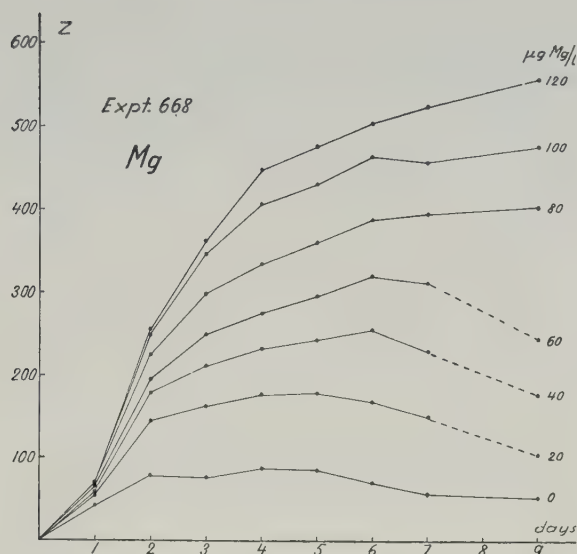


Fig. 25.

Growth (extinction values, Z) of magnesium-starved *Scenedesmus quadricauda* for different concentrations of magnesium.]

after autoclaving — 0.5 ml of the algal suspension from a 16 day old magnesium-starved filter tube culture. Magnesium (as MgSO_4) was then added in seven concentrations (with two tubes for each) from 0 to 120 $\mu\text{g Mg/l}$, besides which, distilled water was added for the compensation of volume differences. The tubes were photometrically measured (whereby $e_0 \times 10^3$ was found to lie between 150 and 170) and then placed in a photothermostat at 25°C , with illumination for 20 hours daily and continual shaking. The results of the following photometric measurements (extinction increases, Z) are shown in fig. 25 on the basis of the average values. During the last days of the experiment the algae in the cultures with the three lowest magnesium concentrations adhered to the walls of the tubes (mostly on the bottom), whereby the values in question are not reliable.

Judging from the curves for the first two days 100—120 $\mu\text{g Mg/l}$ seem to be able to give the maximum rate of growth. After a longer time the differences between the yields for the different magnesium concentrations became much more obvious. The relation between the magnesium concentrations and the corresponding highest increases of extinction (after subtraction of the highest extinction value of the control cultures) follows from the figures in Table 25, where the net increase per $\mu\text{g Mg/l}$ is calculated.

For both the lowest concentrations, 20 and 40 $\mu\text{g Mg/l}$, the magnesium content had its greatest relative effect on the substance production. Con-

Table 25. Experiment 668. Calculation of the extinction increase per $\mu\text{g Mg/l}$.
Highest extinction value of the control cultures: 87.

$\mu\text{g Mg/l}$	Highest <i>Z</i> -value	Net increase	Net increase per $\mu\text{g Mg/l}$
20	179	92	4.6
40	256	169	4.2
60	320	233	3.9
80	404	317	4.0
100	477	390	3.9
120	559	472	3.9

sequently the same relation occurs here as for the iron additions (Tables 22 and 24), and here also I interpret this according to the theory that a limiting factor has greater effect the more it exists in minimum in relation to the other factors (cf. p. 112). In the range 60—120 $\mu\text{g Mg/l}$ the relative effect was practically constant, which means that the magnesium content was the deciding factor for the extent of the substance production. If the experiment had been extended to higher magnesium concentrations, on the other hand, first the nitrate and then the phosphate would have begun to come in the minimum. In the complete culture solution VIII the magnesium content is 1 mg/l, which must be sufficient for considerably higher yields than can be obtained with the nitrate and phosphate contents of this medium.

The effect of different potassium concentrations on the growth was investigated in experiment 742, which was arranged and carried out in the same way as the previous magnesium experiment. It was inoculated with a potassium-starvation culture, which had been allowed to grow for a good two months in a filter tube with culture solution VIII ($\text{K} = 0$) which was changed three times each week. Potassium (as KCl) was graded from 0 to 1,000 $\mu\text{g K/l}$. The initial values ($e_0 \times 10^3$) of the different cultures varied between 217 and 246. The course of the increase of the extinction is shown in fig. 26.

One notices especially that some of the curves show negative values, i. e., a diminution of the extinction in relation to the initial value. This holds especially for the control culture, which during the whole time of the experiment had a lower extinction than at the start. The culture with 20 $\mu\text{g K/l}$ reached positive *Z*-values after six days, while the cultures with 60 and 80 $\mu\text{g/l}$ did so after three and two days respectively. The remaining concentrations gave an increase of the extinction from the very beginning.

It seems to be difficult to interpret these facts in any other way than

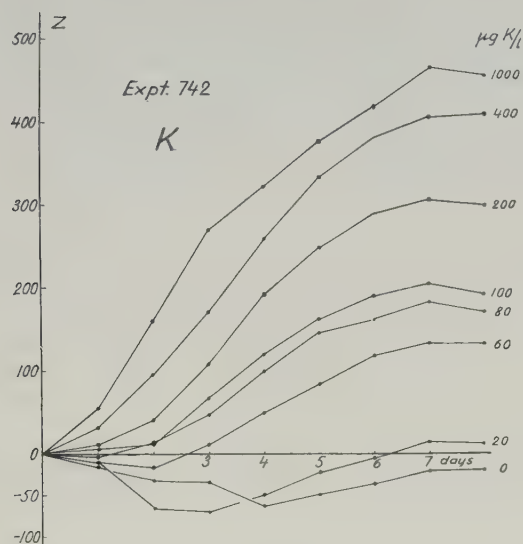


Fig. 26.

Growth (extinction values, Z) of potassium-starved *Scenedesmus quadricauda* for different concentrations of potassium.

that a part of the added amount of potassium was used to cover a deficit due to the long potassium starvation, and that this part could not therefore be utilized directly for increased substance production. The covering of this deficit resulted in the relative effect of the potassium additions being considerably less for the lowest potassium addition than for the nearest higher concentrations (Table 26). In this respect we can thus establish a difference between potassium starvation on the one side, and iron and magnesium starvations on the other (cf. Tables 22, 24, and 25). Concerning phosphate and nitrate starvations, data of this kind are not yet available, but expts 840 and 929 (pp. 76 and 94) seem to indicate that their resulting cellular debts are resembling the potassium debt in the taking up of a certain amount of a given supply (of phosphate and nitrate respectively) without a corresponding increase of growth. Thus the law of relative effects does not hold for a limiting factor, if a previous starvation with regard to this factor has caused a cellular debt which must be satisfied before the growth can start again. Biochemical investigations concerning the starvation effects of different nutrients should certainly give results of great interest.

The marked decrease of the relative potassium effect at 0.4 and 1.0 mg K/l must depend on other factors (especially the nitrate and the phosphate) beginning here to come in the minimum. In culture solution VIII

Table 26. Experiment 742. Calculation of the increase of the extinction value per $\mu\text{g K/l}$ after 7 days. The value of the control culture ($Z = -22$) has not been subtracted.

$\mu\text{g K/l}$	Z	Z per $\mu\text{g K/l}$
20	14	0.70
60	134	2.23
80	182	2.28
100	205	2.05
200	304	1.52
400	407	1.02
1,000	463	0.46

the potassium concentration is 2.2 mg/l; potassium can consequently scarcely become a limiting factor for the growth of *Scenedesmus quadricauda* in this medium.

In lakes of various kinds, of course, the concentrations of magnesium and potassium can be quite different. One can give as the most usual range of concentration for magnesium 0.5—10 mg/l, and for potassium 0.2—3 mg/l (cf. LOHAMMAR 1938). Consequently the magnesium content of lakes practically always lies over the lower limit of even the (assumed) potential magnesium optimum for *Scenedesmus quadricauda*. On the other hand their potassium content can be lower than the potential potassium optimum, but owing to the lack of phosphorus and nitrogen it must always be over the lower limit of its actual optimum. Concerning *Scenedesmus quadricauda* I can thus confirm the common conception that magnesium and potassium should not be counted with the factors which limit the algal development in lakes.

IX. Temperature and Light as Factors for the Growth and Annual Periodicity of Plankton Algae.

Introduction.

The question of the causes for the periodic changes, "pulses," in the development and composition of phytoplankton is as old as plankton research itself. It is not possible here to refer in detail to the different theories which have been submitted to explain the most often regular — but not seldom apparently erratic — course in the annual periodicity of plankton algae. Such an account would give a reflection of an essential aspect in the history of limnology and is worth an independent treatment. I must be content here to sketch quite briefly the point of view — or rather, concerning the causes, the lack of a unitary point of view — which seems to be valid at present in questions on the phytoplankton periodicity.

One knows that the plankton production as a whole, in numerous lakes, traces out a bimodal curve during the course of a year (cf., e. g., PENNAK 1946, fig. 2) with a spring and an autumn maximum and an intermediate summer minimum, a rule which has however many exceptions. Furthermore one knows (see WELCH 1935 p. 237, PENNAK 1946 p. 343, and many original papers) that the diatoms often enter upon high production during the spring and autumn months (sometimes, however, also in winter, even under the ice, more seldom in midsummer); this is the foremost cause for the bicyclic form of the total curve. On the other hand green algae have their best time during the summer months but they are often succeeded towards the end of the summer by highly productive planktonic Cyanophyceae. The Chrysophyceae generally prefer spring or simply winter, while Dinophyceae (Peridineae) most often have their maximum in the summer.

This sketch is, of course, incomplete and to a large extent schematic, all the more so as the periodicity is by no means uniform within the taxonomic algal groups. Besides, there are many plankton algae which do not show any pronounced production pulse. Nevertheless, on the whole, the given characteristic should agree with the average conditions in reality.

More serious difficulties, however, appear immediately when one proceeds from establishing facts to seeking their explanation. Each conceivable factor or circumstance has been examined with respect to its possible correlation to the changes of the phytoplankton production. Most debated have been the causes of the bimodality of the bulk of the planktonic diatoms. So far the discussion does not seem to have given a definite result. "It does not seem possible to designate any single factor or influence as the sole cause of the periodic maxima and minima. Doubtless, they are the result of the interplay of a combination of factors acting at different intensities. There are still reasons for considering the multiple events concerned in the overturns as having some intimate relations to the maxima in lakes manifesting thermal and chemical stratification. It does not necessarily follow that the annual maxima and minima in all kinds of waters are due to the same combination of causes" (WELCH 1935 p. 242).

The spring and autumn circulations have without doubt a very great importance for the supply of nutrients in the epilimnion and it can therefore be plausible to see in them the foremost cause for the usually — but by no means always — approximately simultaneous increase in the individual numbers of the diatoms (see, e. g., PEARSALL 1923). This point of view seems to a certain degree to have limited the discussion, so that it is often mainly concerned with the possible role of the nutritive factors in the appearance and disappearance of a diatom maximum. On this basis it is, however, particularly difficult to find a plausible explanation for the spring maximum not seldom coming to an end before the nutrients have been consumed (see, e. g., PEARSALL, GARDINER & GREENSHIELDS 1946 p. 16). The chemical factors alone cannot thus be decisive.

The roles of the temperature and light conditions in this connection have of course been the object of many discussions. As early as 1904 WESENBERG-LUND (p. 82) found that "in contrast to the maximum of the Cyanophyceae all the larger maxima of diatoms occur at low temperatures, usually at a temperature which is below 10° C and never above 16° C, except for *Asterionella*; we know of no examples in our lakes of diatoms having reached a larger maximum at the highest temperature of the water." According to his idea it is "obvious that the factor which, in the first instance, decides the development of the numerous and great diatom maxima in a lake, is the temperature of the water." (p. 84, from the Danish).

Since this, many similar observations have been made, but it has sometimes been difficult to correlate both the maxima with the course of the temperature, because they often do not take place at the corresponding temperature levels in spring and autumn: the spring maximum not seldom appears at a lower temperature than the autumn maximum. For an ana-

logous reason it is not possible always to get a direct connection with the light factor, but the difference then has the opposite sign: at the spring maximum the light intensity is usually higher than at the autumn maximum. It is therefore not curious that on the basis of the observations in nature it has been difficult to find a unitary point of view.

Despite this difficulty FINDENEGG, on the basis of many years' investigations in a dozen lakes in Carinthia (Kärnten, Austria), has recently (1943) carried the question of the causes for the periodicity of the plankton algae a long step nearer its solution. He had the successful idea to coordinate the light and temperature dependence of the algae according to the following scheme (loc. cit., p. 389).

	Schwachlichtformen:	Starklichtformen:
Kälteformen:	Winterplankter	Frühjahrsplankter
Wärmeformen:	Herbstplankter	Sommerplankter

Thus assuming the effects of light and of temperature to interfere and to have different optimum levels for different algae, FINDENEGG succeeded in giving simple and, on the whole, convincing explanations for not only temporal variations but also for regional differences of several plankton algae in the investigated lakes. The varying transparencies of the lakes, their temperature differences (primarily dependent on different altitudes), and their stratification conditions are shown even to have a selective effect on the composition of phytoplankton. FINDENEGG by no means disregards the role of the chemical factors for the phytoplankton development, but the concluding part of his paper, where these questions are presumably treated in detail, does not seem to have been published yet.

In my own investigations on the physiology and ecology of different plankton algae I have not yet been able to carry out the planned programme of work concerning the temperature and light factors. However, some preliminary experimental results in combination with field observations appear to give a contribution to the elucidation of the causes of the phytoplankton periodicity. Further I hope to show that the connections between algal growth and temperature and light conditions are essentially clarified by the recent results of STÅLFELT (1939 a, 1939 b, 1945) concerning the interfering effects of these factors on the carbon dioxide assimilation.

The temperature and light requirements of *Melosira islandica* subsp. *helvetica*.

At the end of November 1943 *Melosira islandica* O. MÜLL. subsp. *helvetica* O. MÜLL. formed an immense production in Erken. From a water-

sample one *Melosira* thread was pipetted into each of six autoclaved culture tubes, containing 10 ml of culture solution Chu No. 10 (CHU 1942 p. 298) +1 ml of soil extract. The tubes were placed in pairs in three photothermostats (see ÅBERG 1943 pp. 27—29) with temperatures of 5° C, 10° C, and 20° C respectively. The illumination, which for each thermostat was made up of an incandescent lamp of 500 W and a mercury lamp of the type HgH 1,000, was switched on for 10 hours daily; the light intensity was about 1,700 lux at the level of the cultures.

After only three days the differences in growth in the cultures of the three thermostats were very obvious. At 5° C each thread had increased in length by about 50 %, at 10° C by about 100 %, but at 20° C by 300—400 % of the original length. At the last mentioned temperature the threads were, however, in contrast to the threads grown at 10° C and 5° C, very pale and had conspicuously extended cells.

Six days after the start the following result was noted: at 5° C and 10° C the threads had continued to grow quite normally and the greatest growth was at 10° C (a direct measurement was impossible, because during growth the inoculated thread had often intertwined into a ball). On the other hand at 20° C the growth had ceased, obviously quite soon after the previous observation, and the threads were dead; study with a microscope showed that their cells were empty or had a light and granular content.

After two weeks the growth of *Melosira* had ceased at 10° C also, while the tube at 5° C was full of very fine *Melosira* coils. The experiment had to be stopped here owing to *Chlorella* and other Chlorococcales appearing in an increasing production.

In order to check this experiment I placed, one week after its start, three litre flasks of Jena Geräte glass with surface water from Erken (which, after the sampling, had been kept in a dark refrigerator at 4° C) by the side of the culture tubes at 5° C, 10° C, and 20° C respectively. Next to the dominating *M. *helvetica*, *Asterionella formosa* was particularly common in the flasks.

The development of *M. *helvetica* just sketched was repeated in full in the three flasks. At 20° C a very intensive growth started during the first days of the experiment with the formation of abnormally long and hyaline cells, which then quickly died. At 5° C and 10° C the growth was normal and fine, first with the emphasis on 10° C but then (after one week) best at 5° C. On the other hand *Asterionella* gave the best growth at 10° C and 20° C. The experiment could not be extended much longer than one week since the "weed flora" then began to take the upperhand.

We shall now try to get a physiological basis for the continued discussion concerning the importance of the temperature and the light for the

growth of *M. *helvetica*. The growth stands, of course, in intimate relation to the assimilation of carbon dioxide. By means of assimilation experiments with the lichen *Usnea dasypoga*, STÅLFELT (1939 a and b) found that for both light and temperature one must distinguish between on the one hand a fixed lower "limit optimum" (Grenzo optimum), and on the other hand a higher situated movable optimum. By limit optimum is meant that value at which the inhibiting effect begins to set in. The mobility of the other optimum depends on the light and temperature inhibitions increasing with time, whereby the optimum level is depressed more and more the longer the exposure.

The limit optimum of the light for the experimental object used by STÅLFELT lay so low that it could not be established. The movable optimum therefore moved in the whole of the investigated light range (32,000 to 2,000 lux) and was dependent on the light inhibition, which in its turn was a function of both light intensity and time (1939 b, pp. 390 and 392). The light inhibition was independent of the temperature.

The limit optimum of the temperature was found to lie relatively high, at about 21° C, and it was independent of the light factor and the time. STÅLFELT could not experimentally establish a higher, movable temperature optimum, which he attributed to his long experiment times (two hours or more). The temperature inhibition, beginning at the limit optimum and increasing for higher temperatures, became more and more marked the higher the light intensity. Thus the interplay of temperature and light is governed, even with respect to its negative effects, by the law of relative effects (cf. p. 112).

Below the limit optimum of the temperature only the light can have an inhibiting effect, above this value the decreasing assimilation is a consequence of the negative effects of both the light and the temperature. The light intensity then receives a double importance, since it affects not only the light inhibition (if the light intensity is above its limit optimum) but also the extent of the temperature inhibition.

With regard to these experiences of STÅLFELT, the light and temperature dependence of plankton algae must with great probability be considered as a complex function, where the effects of the light and the temperature — positive and negative — are intimately correlated to each other. Therefore, one violates nature by trying to isolate completely the effect of the light factor from that of the temperature or vice versa. Furthermore, the limit optimum of the temperature (the level below which the temperature has only a positive effect) appears to be of great importance for the influence of a certain temperature-light combination. If, of two plankton algae with equal light requirements, species A has its temperature limit optimum at

20° C while species B has this at 10° C, then an environment at 15° C with a certain light intensity above the limit optimum involves quite different conditions of growth for both algae not only with respect to the temperature. For species A exists at the most a "simple" light inhibition (which perhaps is more than compensated by the positive effect of the light), while species B is probably exposed to a twofold negative effect from the side of the light: on the one hand the direct light inhibition, on the other the reinforcement by the light of the temperature inhibition. The difference should become particularly obvious for the two algae if the temperature increases to, e. g., 17° C with a simultaneous increase of the light intensity, i. e., a course which takes place in our lakes each spring and early summer.

It must further be of great ecological importance that the actual optimum of the light is movable, dependent on both the time and the light intensity. Thus, this optimum is not an independent constant, as generally supposed. The temperature also probably has a movable optimum, the position of which is determined by the duration of the temperature inhibition. The experiments described above confirm that the movable optima are a reality for the growth of the plankton algae. At the beginning of the experiments *Melosira *helvetica* gave the quickest (although somewhat abnormal) growth at 20° C and 1,700 lux; by means of continued exposure these conditions caused even the death of the algae. After a few more days a diminution of the growth could be observed even at 10° C, while the growth was still very good at 5° C.

The annual periodicity of *Melosira *helvetica* in Erken and its average temperature and light conditions.

Melosira islandica subsp. *helvetica* occurs particularly in eutrophic lakes, where in spring and autumn it is often a dominant component of the phytoplankton. During midsummer, on the other hand, it is generally completely or nearly completely missing in the epilimnion, but it then occurs in the hypolimnion if a stable stratification is formed. This holds, e. g., according to ASTRID CLEVE-EULER (1912 p. 239), for Lake Mälaren. I can state the same result on the basis of eight years' observations of phytoplankton in Erken. In fig. 27 are reproduced the frequency reports (cf. p. 88) for *M. *helvetica*, recorded by SKUJA in connection with his taxonomic treatment of my plankton samples (net samples in the surface layer). In the same figure are given the corresponding surface temperatures (ice covering is marked by a bracket over the temperature value).

	III	IV	V	VI	VII	VIII	IX	X	XI
1941		7.3 •	11.6 ≡	17.7 :	21.3	18.2		6.7 =	
1942		17 =					17.6		5.9 ≡
1943		3.9 =	7.7 =	17.1	15.9 18.5	12.7 :	15.3 :	10.6 =	8.7 ≡ 3.8 ■
1944			4.4 10.3 — =	14.9 =	20.6 :	18.2 19.5	15.4 14.2	12.4 10.8 — =	7.4 5.2 — •
1945		3.3 6.4 : —	9.0 13.0 : —	15.0	20.6 23.6	17.5 19.6	14.8 14.4	10.6 7.6 : :	5.1 •
1946	7.4 •	5.2 —	13.3 14.4 : •	16.9	18.5	19.8 18.2 •	17.3 15.6 —	11.4 9.5 : =	5.7 2.6 ≡ =
1947				16.0 14.0	21.2 18.5 22.0	19.0	16.9 15.5	12.0 8.5 :	4.5 1.5 : =

Fig. 27.

Frequencies of *Melosira islandica* subsp. *helvetica* and temperatures in the surface water of Erken, March—November, 1941—1947.

From fig. 27 it is clear that *M. *helvetica* in Erken gives fairly regularly one maximum in April and May and one in October and particularly November (usually much higher than in the spring). During June, July, and August it does not occur at all, or but seldom, in the plankton samples and even in September it is often completely lacking (e. g. 1944, 1945, and 1947). The occasions when it is met in the epilimnion during the summer months have nearly always been preceded by a partial or total intermediate circulation (caused by cold and windy weather) whereby the alga is conveyed to the surface from the hypolimnion, where it always occurs throughout the summer. The autumn circulation usually begins in Erken during the last half of August; we notice that the autumn maximum of *M. *helvetica* never begins in association with this.

In order to give an idea of the average light and temperature relations in Erken, I can quote the following from my observational material from the years 1940—1947.

The water is relatively clear and colourless. The colorimetric measurement with K_2PtCl_6 generally gives values between 14 and 20 mg Pt/l for the surface water. My transparency values from the ice-free period (Secchi

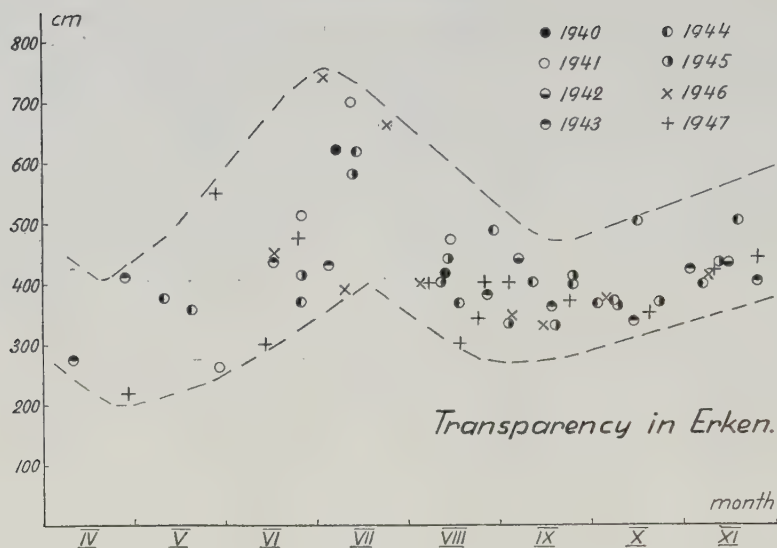


Fig. 28.

Transparency in Erken, April—November, 1940—1947.

disc of 25 cm diameter) are given graphically in fig. 28, which shows that the variations from year to year at a certain time can be fairly large (differences up to 2 m or somewhat more) but that despite this a definite yearly course can be distinguished. Thus the transparency is lowest during spring and autumn with an intermediate maximum during midsummer (high transparency also during winter under the ice).

The principal features of the spectral light absorption of the surface water are shown in Table 27 where the results of some photometric measurements at different seasons of 1941 may be compared. The low extinction coefficients and their mutual relations show that brown (humus) substances do not play any noteworthy role for the colour of the Erken water. The spectral distribution agrees fairly well with that which ÅBERG & RODHE (1942 p. 66) established in Lake Fiolen. Furthermore it may be seen that the extinction values are highest in spring (28th May) and in autumn (14th August and 27th October) and considerably lower in midsummer (11th July); in 1941 the water was obviously almost as clear and colourless in July as under the ice-layer (10th April). I have found this course of the extinction values repeated for other years. It agrees completely with the annual variations of the transparency mentioned above.

For the calculation of the subaquatic light climate, actinometric determinations of the intensity and spectral distribution of daylight at different

Table 27. Extinction coefficients for unfiltered surface water from Erken 1941.
(Under the indication of the colour filter is given the approximate optical centre in $m\mu$ for the combination photoelement + colour filter; cf. ÅBERG & RODHE 1942 p. 11.)

1941	BG 5 460	VG 9 530	RG 1 640	RG 8 740	RG 7 790
April 10 (ice!)	0.83	0.31	0.61	1.99	2.84
May 28	2.18	1.25	1.30	2.57	3.78
July 11	0.92	0.32	0.54	2.00	3.29
August 14	1.19	0.53	0.77	2.10	3.50
October 27	1.44	0.75	1.14	2.50	3.88

Table 28. Erken, July 11th 1941, 12:45—13:00 (sun, cloudless, almost calm).

Depth m	BG 5	
	%	ϵ
(above surface)	100.0	
0.1	67.5	1.10
1	25.0	0.74
2	12.0	0.72
3	5.9	0.76
4	2.8	0.77
5	1.3	0.64
6	0.7	

depths and at different times of the day and year would be of great value. However, from the ice-free period I only have at my disposal one series (11th July 1941), and owing to a mishap only the measurements with colour filter BG 5 (optical centre 460 $m\mu$) could be carried out (Table 28). This series shows great similarity with the corresponding ones from two complete measurements in Fiolen (ÅBERG & RODHE 1942 pp. 40 and 41). It seems therefore to be justified in making these Fiolen series the foundation for an approximate calculation of the decrease of light in Erken, all the more so as the surface water of both the lakes, as has just been pointed out, seems to be very similar with regard to the spectral absorption of the light. By using the method of calculation given by ÅBERG (1943 pp. 95—100), it is found that of the total light radiation incident on the water surface approximately 40 % reaches down to a depth of 1 m, 25 % to 2 m, 10 % to 3 m, and 5 % to 4 m. These values relate to the water in Erken on July 11th, 1941. On this occasion, however, the Erken water was unusually clear (transparency 7 m, see also the low extinction values in Table 27). Hence

Table 29. Calculation of the mean monthly values for the average light intensity in lux for the neighbourhood of Stockholm (approximate values).

Refer to the text.

	IV	V	VI	VII	VIII	IX	X	XI	XII
$E_s H_{akt.}$	4.3	7.0	8.3	7.6	5.8	3.6	1.5	0.5	0.2
H	14.3	17.0	18.3	17.7	15.5	12.8	10.3	7.7	6.3
$E_{s, akt.}$	0.30	0.41	0.45	0.43	0.37	0.28	0.15	0.07	0.03
lux	30,000	41,000	45,000	43,000	37,000	28,000	15,000	7,000	3,000

Table 30. Approximate mean monthly values for the average light intensity (lux, calculated) and the temperature ($^{\circ}\text{C}$, according to observations 1940—1947) in the surface layer of Erken during the ice-free period.

Depth m	IV	V	VI	VII	VIII	IX	X	XI	XII
1	12,000	16,400	18,000	17,200	14,800	11,200	6,000	2,800	1,200
2	7,500	10,250	11,250	10,750	9,250	7,000	3,750	1,750	750
3	3,000	4,100	4,500	4,300	3,700	2,800	1,500	700	300
4	1,500	2,050	2,250	2,150	1,850	1,400	750	350	150
$^{\circ}\text{C}$, 0 m	5	8	15	19	18	15	10	5	2

it must be assumed that, particularly during spring and autumn, the light decreases more quickly from 0 to 4 m than given by these calculated percentage values.

As unit of illumination (E_s), AURÉN (1930 p. 9) has used the average value for the illumination from sun and sky on a horizontal surface at 45° solar altitude, clear sky and free horizon. 1 $E_s H$ is defined as the quantity of light which, during one hour at a light intensity of 1 E_s , irradiates each cm^2 of the surface. Taking into consideration the cloudiness and number of hours of light, AURÉN (1939; see also ÅBERG 1941 p. 31) has determined the average actual amount of light per day ($E_s H_{akt.}$) in mean monthly values, for the neighbourhood of Stockholm. These values are reproduced in my Table 29 for the months April—December. By dividing them by the mean monthly values for the length of the days (H, according to the calendar), I have obtained the mean monthly values for the average actual light intensity ($E_{s, akt.}$). According to AURÉN (1930 p. 17), we can approximate 1 E_s to 100,000 lux (available determinations are not completely in agreement, but the order of magnitude should be about this), whereby we can

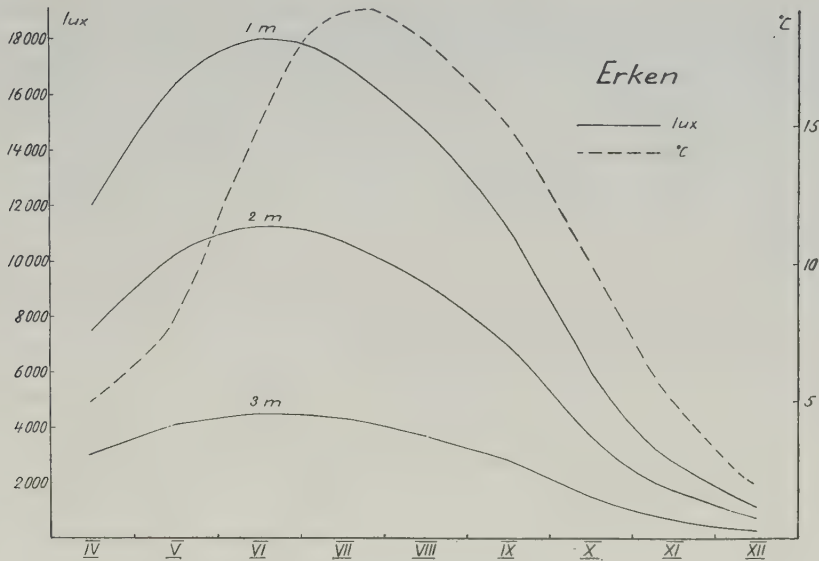


Fig. 29.

Average light intensity (lux, calculated) and temperature (°C, observed) in the surface layers of Erken during the ice-free period of the year;

finally calculate the mean monthly values for the average light intensity expressed in lux (Table 29). By multiplication of these values by 0.40, 0.25, 0.10, and 0.05, the values in Table 30 of the mean monthly values of the average subaquatic light intensity at depths of 1 m, 2 m, 3 m, and 4 m in Erken have been obtained. In the same table is also given the approximate mean temperature of the surface water for each month during the ice-free period according to my measurements during the years 1940 and 1947 (see fig. 27). Table 30 is graphically represented in fig. 29.

It may first be pointed out that the lux value is a very approximate and incomplete measure of the light intensity. The energy of daylight is unequally distributed in the spectrum and the composition of the light is altered very considerably on radiating into a lake. This alteration cannot be expressed in the calculated lux values for different depths. Their use can, however, be justified in a case like this, where it is only intended to get an idea of orders of magnitude.

We may further make it clear that the light curves in fig. 29 represent the course of the average light intensities (for the hours of daylight) at depths of 1, 2, and 3 m in Erken during the ice-free period of the year. The maximal intensity (at midday) is about 50—75 % greater than the average, if one counts upon the same conditions of cloudiness; for clear

sky and maximum sun elevation the light intensity can probably exceed the values given in Table 30 by at least 100 %, if the water is as clear as for the measurements of July 11th, 1941. This is, however, certainly never the case during the spring and autumn months, whence the average curves should lie too high for these months. On account of all these uncertainties the lux values and the illumination curves can only be used to give a rough idea of the orders of magnitude and of the principal course of the sub-aquatic light conditions in Erken.

With these reservations in mind we examine, on the ground of figures 29 and 27, the temperature and light conditions in Erken during the annual maxima of *Melosira *helvetica*. With the exception of the fairly rich occurrence at the end of June 1944, which was caused by an intermediate summer circulation carrying algae from the lower layers to the surface layer, high productions have only been noted before the beginning of June and after the beginning of October, i. e., at temperatures lower than 13° C. The light intensity at a depth of 1 m at the spring maximum is less than about 18,000 lux and at the autumn less than about 8,000 lux. At the end of November, when, in 1943 especially, an enormous production develops, the light intensity at a depth of 1 m is only of the order of magnitude 2,000—3,000 lux at a temperature of about 4° C. As a matter of fact the average light intensity which each individual *Melosira* thread can receive must, however, be considerably less than the values given. During the periods of the maxima (with the possible exception of the end of May) the lake is in total circulation, whence the algae must be assumed to be in almost continuous movement between the surface and the bottom (18.5 m in the deepest place).

During the summer months, when *Melosira *helvetica* is practically lacking in the upper water layer, the surface temperature exceeds 15° C and reaches on an average at the end of July up to about 19° C (maximum temperatures of more than 25° C have, however, been recorded in July for some years). At a depth of about 1 m the average light intensity culminates with about 18,000, at a depth of 3 m with about 4,000 lux.

Comparison of the experimental and ecological data.

In the experiments described above (p. 126) *M. *helvetica* grew well at 5° C and 1,700 lux but at 20° C and the same light intensity the alga died within a week. Consequently the temperature factor alone gives sufficient cause for *M. *helvetica* to avoid the upper layers of the temperate lakes during the summer months. In lakes which do not get a stable summer

stratification with a cooler hypolimnion, the alga may have difficulty in existing from year to year. To a large extent this can be confirmed by field observations. Thus, the species is lacking, according to the investigations of both A. CLEVE-EULER (1922) and myself, in the lake Säbysjön (only 2.4 m deep) south of Uppsala. In the adjacent lake Valloxen, the outflow of which goes into Säbysjön, I have on the other hand found *M. *helvetica* (Oct. 1942 and Nov. 1945). Valloxen is maximally about 12 m deep, and has a stable summer stratification in its deeper parts. With regard to the chemical factors, Säbysjön and Valloxen seem to be in close agreement. This is therefore a fairly clear case of the temperature acting as a selective factor on the composition and regional distribution of the phytoplankton (cf. FINDENEGG 1943 p. 396).

To compare directly the light intensity of the experiments with the light intensities in Erken is very hazardous, especially if the comparison is based on lux values. Certainly a combination of incandescent lamp and mercury lamp is used in the photothermostats, whereby a spectral intensity distribution is obtained which corresponds to that of daylight better than incandescent lamps alone or mercury lamps alone. Despite this, however, the qualitative differences are so great that the lux values can only be used to give a rough idea of the orders of magnitude.

In the uppermost water layers of Erken (0—2 m) an average light intensity of about 1,700 lux exists during the vegetation period only in November (Table 30, fig. 29). Just at this time of year the highest productions of *M. *helvetica* are noted. Since the algae, on the basis of vertical convection and circulation of the water, certainly cannot then limit their occurrence to the surface layer, one should count upon the available light intensity on the average being considerably less than the order of magnitude given. One must inevitably admire the ability of *M. *helvetica* to utilize small amounts of light for a very intensive development.

As pointed out above, the light intensity is considerably higher at the spring maximum than at the autumn maximum. Further, the daily period of illumination is about twice as long during April—May as during October—November (Table 29; H). The temperature, on the other hand, is lower in May than in October (Table 30). The courses of temperature, as well as of light intensity and daily length of illumination, increase in spring but decrease in autumn. We have now to find out whether these environmental differences may be decisive for the spring maxima of *M. *helvetica* being usually lower than its autumn maxima.

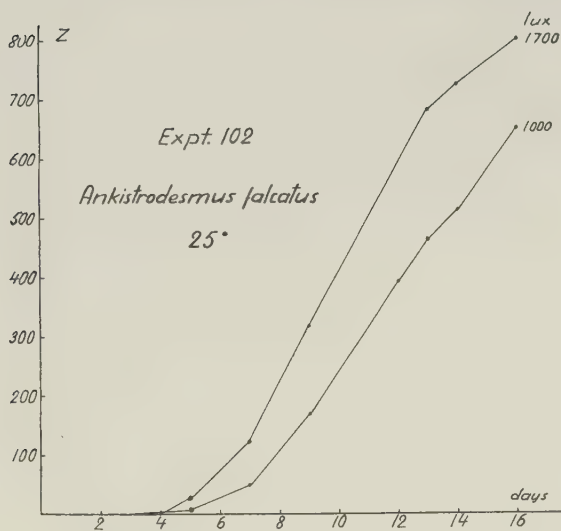
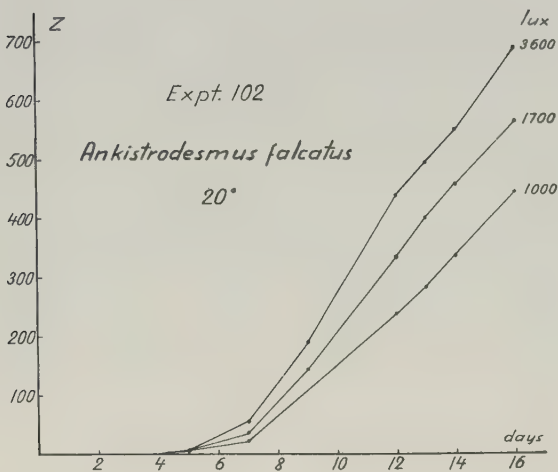
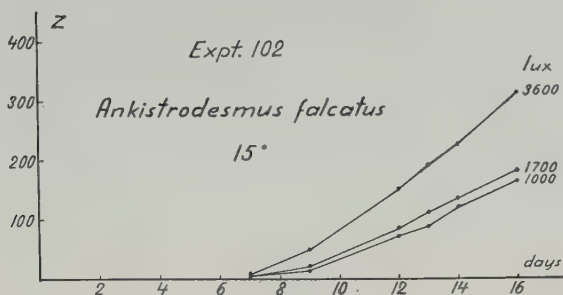
With the guidance of the experimental results we can assume with great certainty that the limit temperature optimum of *M. *helvetica* lies fairly low, perhaps at about 5° C. The more the water temperature in spring

risers above this level, the more the temperature inhibition increases. At first, however, it probably does not assert itself with regard to the production, since the positive effect of light is dominating and gives high yields. But the continual increase of the light intensity raises the negative light effect, which is furthermore reinforced by the increases of both the length of daily illumination and the temperature. Thus the combined increasing negative effects of both temperature and light are, in my opinion, the main cause for the spring maximum of *M. *helvetica* not generally reaching the same height as the autumn maximum. Further, in spring and early summer the inhibiting effects should be strengthened by the formation of a temperature stratification; in the epilimnion the algae are more exposed to high intensities of light and rapidly rising temperatures than they are when the whole water mass is circulating. The development of *M. *helvetica* therefore finally ceases in the upper layers, but during the summer the algae find a retreat in the hypolimnion.

In autumn the inhibiting effects of temperature and light are perpetually decreasing. A light intensity corresponding to that of May is passed as early as in the beginning of August, but the temperature is then still too high (about 18° C) for *M. *helvetica*. Even in the first weeks of October the temperature (about 12° C) is higher than after the culmination of the spring maximum. Nevertheless good growths are observed at this time. This is quite understandable, according to the idea put forward above, since the light intensity of October cannot have as strong negative effects as the much higher illumination in spring. In late November, when neither light nor temperature can be supposed to have any negative effects at all, *M. *helvetica* generally reaches its highest standing crop of the year.

Note on the temperature and light requirements of *Ankistrodesmus falcatus* and other plankton algae in relation to their annual periodicity.

Even in my first culture experiments with various plankton algae I observed that the Chlorococcales investigated appeared to have completely different light and temperature requirements from *Melosira *helvetica*. As example of this may be quoted an experiment (No. 102) with *Ankistrodesmus falcatus* (CORDA) RALFS. Nine identical pure cultures of this alga in culture solution Chu 10 + 10 % soil extract were placed in three photo-thermostats at 15° C, 20° C, and 25° C at three different distances from the light source; the light intensities for these levels were approximately 3,600, 1,700, and 1,000 lux respectively, the time of illumination 10 hours per



Figs 30 A—C.

Growth (extinction values, Z) of *Ankistrodesmus falcatus* at 15° C (fig. 30 A), 20° C (fig. 30 B), and 25° C (fig. 30 C) with different light intensities (1,000, 1,700, and 3,600 lux).

day. The growth was registered by the determination of the increase of extinction (Z). The results are shown in figs 30 A—C.

We see that rising temperature up to at least 25° C had a favourable effect on the growth. For the experimental time used a three to four times higher yield was obtained at 25° C than at 15° C. Also, increase of light to at least 3,600 lux caused an increase of the growth.

Ankistrodesmus falcatus never becomes a dominant in the lakes in the manner of, e. g., *Melosira *helvetica*. Its rather high requirements of phosphorus and nitrogen are in the first place answerable for this (cf. expt. 218, p. 47). One can usually establish, however, that the alga is most common during summer. This monocyclic periodicity completely agrees with its temperature dependence, since the increase proved to be considerably more rapid at 25° C and 20° C than at 15° C. *Ankistrodesmus* prefers temperatures which are fatal for *M. *helvetica*.

Expt. 102 gives no information concerning the importance of the light factor for *A. falcatus* under natural conditions, since it was carried out at light intensities which were considerably lower than those occurring during summer in the uppermost layer of the lakes. It is a known fact that this layer during calm weather often contains a smaller number of plankton algae than the nearest deeper layers (we here disregard the Cyanophyceae which, on the ground of their low specific weight, under such conditions rise up to the surface). It has also been shown (see, e. g., JUDAY & SCHOMER 1935, SCHOMER & JUDAY 1935, CURTIS & JUDAY 1937) that the photosynthetic activity of phytoplankton is lower immediately below the surface than a little deeper. One has generally assumed that this depends on the inhibiting effect of the strong light. This explanation appears probable, particularly with regard to the movable light optimum of the plankton algae being lowered by the long daily periods of illumination which occur during midsummer.

It is not only concerning *Melosira *helvetica* and *Ankistrodesmus falcatus* that I have been able to establish good agreement between the most suitable experimental temperature and the average temporal variation of the alga in lakes. None of the cultivated diatoms has proved to be so sensible to high temperatures as *M. *helvetica*, but none of them is so consistently lacking in the epilimnion during the summer months and has such a late and high autumn maximum as this species. Most of them, however, have a bicyclic periodicity, and in agreement with this it has proved in experimental work not to be advantageous to use temperatures above 15° C. As examples of this can be quoted *Asterionella formosa* and *Fragilaria crotonensis*. In experiments with *Synura uvella* (Chrysophyceae), I was obliged to keep the cultures at 5° C, in order that the alga should not

die; this species also in nature reaches its best development at low temperatures, often under the ice or shortly after the break-up of the ice. For Chlorococcales, e. g. species belonging to the genera *Ankistrodesmus*, *Scenedesmus*, *Chlorella*, *Pediastrum*, and *Coelastrum*, one should, however, keep the cultures at 20° C—25° C, if rapid growth is required. In lakes all of these species prefer the summer months.

Discussion.

Temperature and light are without doubt factors of greatest importance for the production of phytoplankton. This has been known for a long time already, but the effect of the two factors on the growth of the different algae may be of a more complicated character than one generally imagines. The individual organisms probably have not fixed temperature and light optima, but their levels may to a certain degree be dependent on the time of exposure. To this is added that the two factors affect each other's effects to a high degree. Their correlation is not of such a simple nature that rising temperature only and always makes possible a better utilization of light for the assimilation of carbon dioxide (cf. RUTTNER 1940 p. 100). The visible response of an alga to a certain combination of factors is a result of the balance between the positive and negative effects of both the factors. A certainly very profitable and rich field of research waits here for experimental investigations. The plankton algae are probably particularly suitable and interesting objects for studies on the physiological and ecological roles of temperature and light.

For the elucidation of the importance of temperature and light for the regional and temporal variations of the plankton algae, however, many difficulties arise. The taxonomic conception of a species (or subspecies etc.) does not represent with certainty a corresponding physiological-ecological unity. One must count with the occurrence of both fairly constant ecotypes (cf. RUTTNER 1937) and more or less accidental adaptations (HARVEY 1939 p. 509). Besides — and above all — the occurrence of the algae in nature is conditioned not only by temperature and light but also by many chemical or biotic factors. We cannot therefore expect the causation between environment and organisms to appear equally obvious in nature as in the controlled experiment. Experimentally we can investigate the effects of the different factors on different plankton algae under certain given conditions. But it is a long step from there to complete understanding of the greatly diversified interplay of factors and organisms, which in the lakes results in an inexhaustible variation of the qualitative and quantitative composition of the phytoplankton.

Summary.

The main purpose of this work is to elucidate the influence of some environmental factors on the development of fresh-water plankton algae. The requirements of some algae, in the first place of *Scenedesmus quadricauda*, were studied by means of quantitative culture experiments under controlled conditions. The experiments were carried out from ecological points of view, and the results are compared with the conditions in lakes.

The contents of the nine sections may be summarized as follows.

I. For different dilutions of the same algal suspension the relation between light absorption and cell concentration is given by a slightly curved line, if the extinction measurements are carried out with non-monochromatic light.

For a growing algal culture the increase of the extinction is rather an expression of substance production than of cell increase. It is consequently impossible to use extinction values as a direct measure of the population density.

The substance production is dependent to a high degree on the light-dark periodicity. Thus in order to obtain comparable extinction values, the photometric determinations must be carried out on occasions which are congruent in relation to such a periodicity.

Substance production, cell multiplication, and chlorophyll formation are rather independent aspects of the development of a culture. Their courses are affected in different ways for a deficiency of, e. g., nitrate. Parallel determinations should therefore if possible be carried out of extinction, cell concentration, and chlorophyll content.

II. $\alpha\alpha'$ -dipyridyl and o-phenanthroline are sensible and reliable reagents for the determination of iron concentrations in water. The commonly used thiocyanate method is less sensible and also considerably more uncertain, especially for analysis of iron in natural waters where the content of reactive iron cannot as a rule be determined with this reagent. This is, on the other hand, possible with phenanthroline.

It is shown by direct analyses with phenanthroline that only a small part of the total iron content of some lake waters exists in reactive form. According to these determinations the content of reactive iron is not proportional to the total iron concentration.

III. In neutral or alkaline solutions of ferric chloride or ferric citrate the concentration of the reactive iron decreases owing to hydrolysis to colloidal ferric hydroxide which easily precipitates. This process is accelerated by autoclaving, and for ferric citrate also by light.

A ferric citrate solution can be stabilized by addition of citric acid. Autoclaving then has no effect on the concentration of reactive iron. In light, the citric acid is decomposed oxidatively by the ferric citrate, the iron of which is partly reduced to the bivalent form. The time of stability is then dependent on the relative amount of citric acid, and the pH. In light, one part citric acid to one part ferric citrate has a stabilizing effect quite sufficient for algal cultures.

IV. On the basis of quantitative growth experiments with *Ankistrodesmus falcatus*, a culture solution (medium VIII) has been composed which has proved to be very suitable for unlimited cultivation of about forty species and forms belonging to Chlorococcales, Volvocales, Conjugatae, Heterokontae, and diatoms.

Plankton algae of mixotrophic character cannot be cultivated in medium VIII. Additions of decoctions of soil or of lake sediment can then have a positive effect in some cases, but not for the planktonic Cyanophyceae. Preliminary experiments with *Gloeotrichia echinulata* in "soil-water cultures" have shown that this alga requires a supply of certain thermally unstable organic substances in order to live.

V. The phosphate dependence of the growth can be very different for various plankton algae.

Scenedesmus quadricauda grows in 10—40 $\mu\text{g P}(\text{PO}_4)/\text{l}$ at only a fraction of the maximal rate. This is attained in about 1,000 $\mu\text{g P/l}$, if the supply of other nutrients is sufficient. The phosphate dependence is the same in completely synthetic solution as in lake water. *S. quadricauda* can utilize phosphorus bound in adenylic acid. With plentiful supply of phosphate the alga accumulates this above the actual need. In phosphate-free medium it can use the cellular phosphorus store for continued growth during several weeks.

Asterionella formosa in inorganic culture solutions has approximately the same phosphate requirement as *S. quadricauda*, but practically no in-

crease is obtained in $10\text{ }\mu\text{g P(PO}_4\text{)/l.}$ In lake water *A. formosa*, on the other hand, can utilize even very small phosphate concentrations, and maximal rate of growth was obtained after addition of only $2\text{ }\mu\text{g P/l.}$ while additions of $50\text{ }\mu\text{g P/l.}$ or more had an inhibiting effect. Lake water is assumed to contain a factor which affects the phosphate dependence of this alga and facilitates its utilization of the phosphate. In addition, however, the physiological condition of the cell is of great importance for the effect of the phosphate on its growth.

Dinobryon divergens and *Uroglena americana*, according to preliminary experiments in lake water, have a very low phosphorus need and their growth is inhibited by additions of only $5\text{--}10\text{ }\mu\text{g P(PO}_4\text{)/l.}$

The supply of assimilable phosphorus has for a long time been considered to be a minimum factor for the phytoplankton development. On the average this is probably true for the standing crop as a whole, since the bulk of plankton algae seems to require more phosphorus than is available in the epilimnion of lakes. The experimental results with *Dinobryon* and *Uroglena* suggest, however, that phosphorus even in ordinary lakes may become a maximum factor for some algae. Ecological observations confirm this suggestion.

VI. The nitrogen requirement of *Scenedesmus quadricauda* is too high to be satisfied by the concentrations available in most natural waters. In culture solutions, where no other nutrients are limiting, a nitrate content of at least 5 mg N/l. is necessary to give unlimited growth and chlorophyll formation for a longer time than a few days.

The cell increase, the substance production, and the formation of chlorophyll are affected by nitrate deficiency to different extents. The chlorophyll formation is most sensible, ceasing shortly after the removal of the nitrate from the solution and then being succeeded by a diminution of the chlorophyll content. The cell increase and the substance production, however, are able to continue for several weeks in a repeatedly renewed solution completely lacking nitrogen, if the cells have previously had a good supply of nitrate.

The relative effect of a given suboptimal nitrate concentration is dependent on the size of the inoculum; the growth and the chlorophyll formation per inoculated cell then being inversely proportional to the initial cell concentration. Thus the competition of algae for available ions must be a factor to be regarded in natural conditions with different algal densities.

VII. If the iron is removed from the solution of a highly productive culture of *Scenedesmus quadricauda* and the iron-free medium is repeatedly

renewed, the cells continue their growth and chlorophyll formation for half a year before stopping.

Addition of as little as 0.5 μg Fe/l has an immediate and significant effect on the growth and the chlorophyll formation of iron-starved *Scenedesmus* cultures. The effects of different iron concentrations on the chlorophyll formation of iron-starved cultures are in agreement with the law of relative effects of limiting factors. Nitrogen in particular, but not phosphorus, interferes with iron as a factor limiting chlorophyll formation. Cell increase and substance production give less definite responses for different iron concentrations.

Iron-starved cultures of *Scenedesmus quadricauda* may be used for biological determinations of the assimilable iron content of lake waters. This method is much more sensible than the phenanthroline analysis. Its application is demonstrated for a lake water sample, showing the content of iron assimilable by *Scenedesmus* to be in agreement with the concentration of iron directly reacting with phenanthroline.

In the epilimnion of most lakes the supply of assimilable iron is less than the optimal iron requirement of *Scenedesmus* in cultures offering suitable amounts of the other nutrients. It is, however, necessary to distinguish between the potential (absolute) and the actual (relative) optimum. Only the former has a fixed position which is dependent on the individual requirement of the organism alone; it is fully realized only when no external factors are limiting and is therefore perhaps of a quite theoretical character. Under real conditions more than one external factor limits the metabolic processes. The actual optimum of each factor is then influenced by the other environmental factors in agreement with the law of relative effects. The level of the actual optimum is more depressed the more other factors are limiting. Therefore, with regard to the insufficient supply of phosphorus and nitrogen, the content of assimilable iron in most lakes is considered to be higher than the actual iron optima of *Scenedesmus quadricauda*.

VIII. The growth and the chlorophyll formation of a magnesium-starved culture of *Scenedesmus quadricauda* cease within a few weeks, while they continue for several months after the removal of potassium from the solution.

Magnesium deficient cells are able to utilize even small amounts of magnesium for immediate growth. In this respect magnesium starvation resembles iron starvation.

Potassium deficient cells, however, require a certain amount of potassium to cover their debt; this amount cannot be used for growth. On ac-

count of this, the effects of suboptimal potassium concentrations on the growth of potassium-starved cultures of *Scenedesmus* do not strictly agree with the law of relative effects. Thus the lowest concentrations of potassium give a smaller relative effect on growth than the nearest higher concentrations. The same seems to hold for phosphorus starvation and nitrogen starvation.

In most lakes the concentration of magnesium is above the lower limit of the (assumed) potential magnesium optimum of *Scenedesmus quadricauda*. Their content of potassium, however, may be lower than the potential potassium optimum, but it is certainly always above the actual optima. Thus in ordinary lakes magnesium and potassium never limit the development of this alga.

IX. *Melosira islandica* subsp. *helvetica* prefers low temperatures. In experiments the best growths were obtained at 5° C and 10° C. During the first days 20° C gave a very rapid but rather abnormal growth, but then the alga died.

In most lakes *M. *helvetica* has a bicyclic annual periodicity with maxima in spring and autumn. In summer it is generally lacking in the epilimnion. Very shallow lakes seem to be avoided altogether. These relations can be explained mainly on the basis of the temperature requirement of the alga.

The actual light optimum of *M. *helvetica* is considered to be movable, its position being dependent on the intensity of light, the length of illumination, and the temperature. In spring the continual increase of the light intensity raises the negative light effect which is furthermore reinforced by the increases of both the daily length of illumination and the temperature. In autumn the course is opposite. The interfering effects of temperature and light in different combinations are supposed to be the main cause of the spring maximum of *M. *helvetica* in the lake Erken being, as a rule, less than the autumn maximum which culminates as late as the end of November.

Experiments with *Ankistrodesmus falcatus* show that an increase of the temperature up to at least 25° C and an increase of the light intensity up to at least 3,600 lux have a favourable effect on its growth. This is in agreement with the occurrence of *A. falcatus* in lakes, where the summer months are preferred.

References.

- ÅBERG, B. 1941. Pflanzenkultur bei künstlichem Licht (Kunstlichtkultur) mit besonderer Berücksichtigung der Tomate. — *Symb. Bot. Ups.* 4 (5): 1—98.
- 1943. Physiologische und ökologische Studien über die pflanzliche Photomorphose. — *Ibid.* 8 (1): 1—189.
- ÅBERG, B., & RODHE, W. 1942. Über die Milieufaktoren in einigen südschwedischen Seen. — *Ibid.* 5 (3): 1—256.
- AURÉN, T. E. 1930. Illumination from sun and sky in the neighbourhood of Stockholm. — *Medd. Statens meteor.-hydrogr. anstalt* 5 (4): 1—26. Stockholm.
- 1939. Radiation climate in Scandinavian Peninsula. — *Arkiv mat., astron. fysik* 26 A (20): 1—50.
- BOLD, H. C. 1942. The cultivation of algae. — *Botan. Rev.* 8 (2): 69—138.
- BOREI, H. 1943. Spektralphotometrische Eisenbestimmung mit o-Phenantrolin und $\alpha\alpha'$ -Dipyridyl. — *Biochem. Z.* 314: 359—372.
- BRYAN, J. M. 1933. The effect of hydrogen-ion concentration on the rate of oxidation of solutions of ferrous citrate. — *Trans. Faraday Soc.* 29: 830—833.
- CHU, S. P. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. — *J. Ecol.* 30: 284—325.
- 1943. The influence of the mineral composition of the medium on the growth of planktonic algae. II. The influence of the concentration of inorganic nitrogen and phosphate phosphorus. — *Ibid.* 31: 109—148.
- 1946. The utilization of organic phosphorus by phytoplankton. — *J. Marine Biol. Assoc.* 26: 285—295.
- CLEVE-EULER, A. 1912. Das Bacillariaceen-Plankton in Gewässern bei Stockholm. II. Zur Morphologie und Biologie einer pleomorphen *Melosira*. — *Arch. Hydrobiol.* 7: 119—139, 230—260.
- 1922. Om diatomacévegetationen och dess förändringar i Säbysjön, Uppland, samt några dämnda sjöar i Salatrakten. — *Sveriges Geol. Undersökn., Ser. C*, 309: 49—77.
- CURTIS, J. T., & JUDAY, C. 1937. Photosynthesis of algae in Wisconsin lakes. III. Observations of 1935. — *Intern. Rev. ges. Hydrobiol. Hydrog.* 35: 122—133.
- DEEVEY, E. S. 1940. Limnological studies in Connecticut. V. A contribution to regional limnology. — *Am. J. Sci.* 238: 717—741.
- EINSELE, W., & GRIM, J. 1938. Über den Kieselsäuregehalt planktischer Diatomeen und dessen Bedeutung für einige Fragen ihrer Ökologie. — *Z. Botan.* 32: 545—590.
- FINDENEGG, I. 1943. Untersuchungen über die Ökologie und die Produktionsverhältnisse des Planktons im Kärntner Seengebiete. — *Intern. Rev. ges. Hydrobiol. Hydrog.* 43: 368—429.

- FLEISCHER, W. E. 1935. The relation between chlorophyll content and rate of photosynthesis. — *J. Gen. Physiol.* 18: 573—597.
- FORTUNE, W. B., & MELLON, M. G. 1938. Determination of iron with o-phenanthroline. A spectrophotometric study. — *Ind. Eng. Chem., Anal. Ed.* 10: 60—64.
- FRANZEW, A. W. 1932. Ein Versuch der physiologischen Erforschung der Produktionsfähigkeit des Moskauflusswassers. (In Russian, German summary.) — *Microbiology (U.S.S.R.)* 1: 122—130.
- Freshwater Biological Association (F.B.A.) of the British Empire. Annual Reports 11—15. 1943—1947. Wray Castle, Ambleside.
- GENDEREN, H. VAN, & SCHOLTENS, R. T. 1947. A photometer for the extincitometric determination of the density of suspensions of microorganisms with minimal deviation from the law of BEER-LAMBERT. — *Antonie van Leeuwenhoek* 13: 153—161.
- GESSNER, F. 1943. Die assimilatorische Leistung des Phytoplanktons, bezogen auf seinen Chlorophyllgehalt. — *Z. Botan.* 38: 414—424.
- 1944. Der Chlorophyllgehalt der Seen als Ausdruck ihrer Produktivität. — *Arch. Hydrobiol.* 40: 687—732.
- GRAN, H. H. 1931. On the conditions for the production of plankton in the sea. — *Conseil permanent intern. exploration mer, Rapports et Procès-Verbaux* 75: 37—46.
- 1933. Studies on the biology and chemistry of the Gulf of Maine. II. Distribution of phytoplankton in August, 1932. — *Biol. Bull.* 64: 159—182.
- GROSS, F., & KOCZY, F. F. 1946. Photometric measurements of the growth of phytoplankton cultures. — *Medd. Oceanogr. Inst. Göteborg* 10: 1—18.
- GROTE, A. 1934. Der Sauerstoffhaushalt der Seen. — *Binnengewässer* 14: 1—217.
- GUSSEVA, K. A. 1938. Hydrobiologic productivity and the prognoses of the florescence of waterreservoirs. (In Russian, English summary.) — *Microbiology (U.S.S.R.)* 7: 303—315.
- GUTHRIE, J. D. 1928. A stable colorimetric standard for chlorophyll determinations. — *Am. J. Botany* 15: 86—87.
- HANDKE, H. H. 1941. Hydrographische und biochemische Untersuchungen über die Plankton-Produktionskraft des Süßen Sees bei Halle. — *Botan. Arch.* 42: 149—200.
- HARVEY, H. W. 1937. The supply of iron to diatoms. — *J. Marine Biol. Assoc.* 22: 205—219.
- 1939. Substances controlling the growth of a diatom. — *Ibid.* 23: 499—520.
- 1945. Recent advances in the chemistry and biology of sea water. — 164 pp. Cambridge.
- HILLE, J. C. VAN. 1938. The quantitative relation between rate of photosynthesis and chlorophyll content in *Chlorella pyrenoidosa*. (Diss., Utrecht.) — *Rec. trav. botan. néerland.* 35: 678—757.
- HOPKINS, E. F. 1930. Iron-ion concentration in relation to growth and other biological processes. — *Botan. Gaz.* 89: 209—240.
- HOPKINS, E. F., & WANN, F. B. 1927. Iron requirement for *Chlorella*. — *Ibid.* 84: 407—427.
- HUTCHINSON, G. E. 1941. Limnological studies in Connecticut. IV. The mechanisms of intermediary metabolism in stratified lakes. — *Ecol. Monographs* 11: 21—60.
1944. Limnological studies in Connecticut. VII. A critical examination of

- the supposed relationship between phytoplankton periodicity and chemical changes in lake waters. — *Ecology* 25: 3—26.
- JUDAY, C., & BIRGE, E. A. 1931. A second report on the phosphorus content of Wisconsin lake waters. — *Trans. Wisconsin Acad. Sci.* 26: 353—382.
- JUDAY, C., & SCHOMER, H. A. 1935. The utilization of solar radiation by algae at different depths in lakes. — *Biol. Bull.* 69: 75—81.
- KETCHUM, B. H. 1939. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. — *J. Cellular Comp. Physiol.* 13: 373—381.
- KOLTHOFF, I. M., & SANDELL, E. B. 1943. Textbook of quantitative inorganic analysis. Revised edition. — 794 pp. New York.
- KOZMINSKI, Z. 1938. Amount and distribution of the chlorophyll in some lakes of northeastern Wisconsin. — *Trans. Wisconsin Acad. Sci.* 31: 411—438.
- KUFFERATH, H. 1929. La Culture des Algues. — *Revue Algologique* 4: 127—346.
- LOHAMMAR, G. 1938. Wasserchemie und höhere Vegetation schwedischer Seen. — *Symb. Bot. Ups.* 3 (1): 1—252.
- LUNDEGÅRDH, H. 1931. Environment and plant development. (Being "Klima und Boden in ihrer Wirkung auf das Pflanzenleben", Jena 1930, translated by E. ASHBY.) — 330 pp. London.
- Metropolitan Water Board, Report for 1938. 1939. — 118 pp. London.
- MÜLLER, F. 1924. Die Blutkörperchenzählung und Bestimmung des Blutfarbstoffgehaltes. — *Abderhaldens Handb. d. biol. Arbeitsmeth.*, Abt. IV, Teil 3: 19—62.
- MÜLLER, H. 1933. Die Verwendung von α' -Dipyridyl zur Bestimmung von Ferro- und Gesamteisen in natürlichen Wässern. — *Mikrochemie* 12: 307—314.
- MÜNSTER STRØM, K. 1933. Nutrition of algae. — *Arch. Hydrobiol.* 25: 38—47.
- NAUMANN, E. 1929. Grundlinien der experimentellen Planktonforschung. — *Binnengewässer* 6: 1—100.
1932. Grundzüge der regionalen Limnologie. — *Ibid.* 11: 1—176.
- ÖSTERLIND, S. 1947. Growth of a planktonic green alga at various carbonic acid and hydrogen-ion concentrations. — *Nature* 159: 199—200.
- 1948. Influence of low bicarbonate concentrations on the growth of a green alga. — *Ibid.* 161: 319—320.
- PEARSALL, W. H. 1923. A theory of diatom periodicity. — *J. Ecol.* 11: 165—183.
- 1932. Phytoplankton in the English lakes. II. The composition of the phytoplankton in relation to dissolved substances. — *Ibid.* 20: 241—262.
- PEARSALL, W. H., GARDINER, A. C., & GREENSHIELDS, F. 1946. Freshwater biology and water supply in Britain. — *F.B.A., Scient. Publ.* 11: 1—90.
- PEARSALL, W. H., & LOOSE, L. 1937. The growth of *Chlorella vulgaris* in pure culture. — *Proc. Roy. Soc. (London), B*, 121: 451—501.
- PELTZ, S. I., & LYNN, E. V. 1938. Decomposition of citric acid by ferric iron. — *J. Am. Pharm. Assoc. Sci. Ed.* 27: 774—775.
- PENNAK, R. W. 1946. The dynamics of fresh-water plankton populations. — *Ecol. Monographs* 16: 339—355.
- PETERS, C. A., & FRENCH, C. L. 1941. A study of the ferric thiocyanate reaction. — *Ind. Eng. Chem., Anal. Ed.* 13: 604—607.
- PIRSON, A. 1937. Ernährungs- und stoffwechselphysiologische Untersuchungen an *Fontinalis* und *Chlorella*. — *Z. Botan.* 31: 193—267.

- PRATT, R. 1940. Influence of the size of the inoculum on the growth of *Chlorella vulgaris* in freshly prepared culture medium. — Am. J. Botany 27: 52—56.
- 1942. Studies on *Chlorella vulgaris*. V. Some properties of the growth-inhibitor formed by *Chlorella* cells. — Ibid. 29: 142—148.
- 1944. Studies on *Chlorella vulgaris*. IX. Influence on growth of *Chlorella* of continuous removal of chlorellin from the culture solution. — Ibid. 31: 418—421.
- PRINGSHEIM, E. G. 1934. Untersuchungen zu USPENSKIS Eisenhypothese der Algenverbreitung. — Planta 22: 267—312.
- 1936. Das Rätsel der Erdbabkochung. — Beihefte Botan. Centr. 55 (A): 100—121.
- 1946 a. Pure cultures of algae. — 119 pp. Cambridge.
- 1946 b. The biphasic or soil-water culture method for growing algae and flagellata. — J. Ecol. 33: 193—204.
- RABINOWITCH, E. I. 1945. Photosynthesis and related processes. I. — 599 pp. New York.
- RILEY, G. A. 1940. Limnological studies in Connecticut. III. The plankton of Linsley Pond. — Ecol. Monographs 10: 279—306.
- RUTTNER, F. 1937. Ökotypen mit verschiedener Vertikalverteilung im Plankton der Alpengseen. — Intern. Rev. ges. Hydrobiol. Hydrog. 35: 7—34.
- 1940. Grundriss der Limnologie. — 167 pp. Berlin.
- SCHILLING, V. 1942. Praktische Blutlehre. 10. und 11. Aufl. — 120 pp. Jena.
- SCHOMER, H. A., & JUDAY, C. 1935. Photosynthesis of algae at different depths in some lakes of northeastern Wisconsin. I. Observations of 1933. — Trans. Wisconsin Acad. Sci. 29: 173—193.
- SCHREIBER, E. 1927. Die Reinkultur von marinem Phytoplankton und deren Bedeutung für die Erforschung der Produktionsfähigkeit des Meerwassers. — Wiss. Meeresunters., N. F. 16 (10): 1—34.
- SCHROPP, W., & SCHARRER, K. 1933. Wasserkulturversuche mit der "A-Z-Lösung" nach HOAGLAND. — Jahrb. wiss. Botan. 78: 544—563.
- SKUJA, H. 1948. Taxonomie des Phytoplanktons einiger Seen in Uppland, Schweden. — Symb. Bot. Ups. 9 (3): 1—399.
- STÅLFELT, M. G. 1939 a. Licht- und Temperaturhemmung in der Kohlensäureassimilation. — Planta 30: 384—421.
- 1939 b. Über die Natur der Licht- und Temperaturoptima in der Kohlensäureassimilation. — Svensk Bot. Tidskr. 33: 383—417.
- 1945. Über die lichtbedingten Hemmungsvorgänge in der Kohlensäureassimilation. — Ibid. 39: 365—395.
- STEEMANN NIELSEN, E. 1947. Photosynthesis of aquatic plants with special reference to the carbon-sources. — Dansk Bot. Arkiv 12 (8): 1—71.
- TEILING, E. 1916. En kaledonisk fytoplanktonformation. — Svensk Bot. Tidskr. 10: 506—519.
- THIEL, A., & HENGEL, E. VAN. 1937. Grundlagen und Anwendungen der Absolutcolorimetrie. XVI. Mitteil.: Über die absolutcolorimetrische Bestimmung des Eisens. — Ber. deut. chem. Ges. 70: 2491—2497.
- THIEL, A., HEINRICH, H., & HENGEL, E. VAN. 1938. Grundlagen und Anwendungen der Absolutcolorimetrie. XVII. Mitteil.: Weitere Erfahrungen mit der Absolutcolorimetrie des Eisens. — Ibid. 71: 756—758.
- THIENEMANN, A. 1941. Leben und Umwelt. — Bios 12: 1—122. Leipzig.

- THUNMARK, S. 1945 a. Die Abwasserfrage der Väckjöseen in hydrobiologischer Beleuchtung. — Medd. Lunds Univ. Limnol. Inst. 4: 1—239.
- 1945 b. Zur Soziologie des Süßwasserplanktons. — Folia Limnologica Scandinavica 3: 1—66.
- TRESSLER, W. L., & DOMOGALLA, B. P. 1931. Limnological studies of Lake Wingra. — Trans. Wisconsin Acad. Sci. 26: 331—351.
- USPENSKI, E. E. 1927. Eisen als Faktor für die Verbreitung niederer Wasserpflanzen. — Pflanzenforschung 9: 1—104.
- USPENSKI, E. E., & USPENSKAJA, W. J. 1925. Reinkultur und ungeschlechtliche Fortpflanzung des *Volvox minor* und *Volvox globator* in einer synthetischen Nährlösung. — Z. Botan. 17: 273—308.
- UTERMÖHL, H. 1925. Limnologische Phytoplanktonstudien. — Arch. Hydrobiol., Suppl.-Band 5: 1—527.
- WELCH, P. S. 1935. Limnology. — 471 pp. New York and London.
- WESENBERG-LUND, C. 1904. Plankton investigations of the Danish lakes. Special Part. I. Text. — 223 + 44 pp. Copenhagen.
- ZAIDES, A. 1935. Interaction of trivalent iron salts with organic acids. (In Russian.) — J. Gen. Chem. (U.S.S.R.) 5: 1530—1536.

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